INTERNAL MEDICINE

Administration of *Astragalus membranaceus* Prevented Kidney Dysfunction in Older Mice Following Renal Ischemia-Reperfusion

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ABSTRACT

Objective: ***Astragalus membranaceus*** **(AM) has been shown to possess various pharmacological effects in several organs including the kidney. However, few findings about the therapeutic effect against experimental acute kidney injury (AKI), at least in part, related to CKD progression.**

Materials and Methods: **C57BL/6 mice were administered with or without AM prior to the left renal ischemia/reperfusion. In advance, about 0.2 ml of blood sample from each mouse was collected by more than two hours before surgery. Twenty hours after the renal ischemia, about 0.2 ml of blood sample was collected from each mouse. These serum samples were measured to detect urea nitrogen (BUN) and creatinine.**

Results: **Various aged mice with unilateral ischemia for 30 minutes showed a slight but significant increase in levels of blood urea nitrogen 20 hours after reperfusion. Interestingly, AM administration normalized this azotemia only in older mice, suggesting a positive AM effect of age-dependent AKI therapy.**

Conclusions: **AM administration can protect kidney against moderate ischemic injury, which would play a critical role in older kidney, indicating that AM administration could reduce day-to-day generated AKI, which is ineffective to younger kidney.**

KEY WORDS

Astragalus membranaceus**, ischemia-reperfusion, acute kidney injury, mouse**

INTRODUCTION

Astragalus membranaceus (AM) is widely used for herbal medicine in Japan as well as in other countries in Asia (*https://nccih.nih.gov/health/astragalus*, Accessed 3 December 2017), and its usage expands into inflammatory diseases, tumors, radical scavenger activity, various cardiovascular diseases, and neuroprotective activity (Auyeung, Han, Ko, 2016; Ho, Jie, 2007; Huang, Ding, Lu, Tang, Deng, Deng, Huang, Tan, Chen, Deng, Huang, Tan, Chen, Deng, 2015a; Huang, Ding, Wang, Qiu, Tang, Zeng, Deng, 2015b; Jia, Leng, Wang, Dai, 2017; Li, Hou, Xu, Liu, Tu, 2017a; Li, Zhao, Wang, 2017b). Especially, about the microcirculatory dysfunction and tissue injury induced by ischemia and reperfusion (IR), AM plays a prevalent role in the cerebrum, small intestine, heart, and so on (Han, Li, Ma, Fan, 2017). For example, evidence has been previously provided regarding the effects of AM and its component on oxidative stress-induced neuronal cell death following cerebral IR (Huang *et al*., 2015a; Huang *et al*., 2015b; Li *et al*., 2017a). Also, AM demonstrated another effect on anti-tumorigenic growth inhibition, apoptotic cell death, and signaling modulation (Auyeung *et al*., 2016; Cai, Li, Wang, 2001; Li *et al*., 2017a). Thus, AM reveals various biological activities dependent on cellular environment. As an important traditional herbal medicine, further studies on AM can lead to the development of new drugs and therapies for various diseases (Auyeung *et al*., 2016; Fu, Wang, Huang, Zheng, Wang, Chen, Zhang, Yang, 2014; Ho *et al*., 2007; Li *et al*., 2017a; Song, Meng, Li, Qu, Li, 2009; Song, Meng, Li, 2008; Tong, Xiao, Yao, Huang, 2014).

On the other hand, it was previously accepted that AKI is transient and presents with reversible loss of kidney function. However, as renal dysfunction increases due to AKI, various damages of kidney structures could also be found as a result of AKI (Rifkin, Coca, Kalantar-Zadeh, 2012). Furthermore, incidence of AKI depends on endogenous susceptibilities such as age and sex (Boddu, Fan, Rangarajan, Sunil, Bolisetty, Curtis, 2017; Kang, Heo, 2015; Silveira Santos, Romani, Benvenutti, Ribas Zahdi, Riella, Mazza do Nascimento, 2017; Wanitsriphinyo, Tangkiatkumjai, 2017). For example, for patients in a worse condition AKI may easily be caused by a short-term exposure such as sepsis (Murugan, Kellum, 2011). However, no effective treatments for AKI are currently available (Murugan *et al*., 2011). Therefore, we strongly suggest that examination of AM to be used as an effective treatment for AKI can lead to new therapies for some kinds of kidney diseases. Here we show an experimental approach with mice for therapies with AM to prevent AKI.

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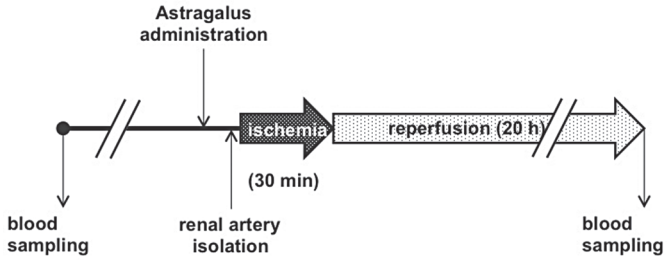

Figure 1. The experimental protocol for investigating the effect of AM administration on renal IR-induced dysfunction. The blood sample from each mouse was collected in advance. Mice receive 0.5% methyl cellulose 400 (vehicle) or AM (50 mg/kg) orally once before IR preparation. A blood sample was collected 20 hours after IR.

MATERIALS AND METHODS

Animals

This study was conducted in accordance with the Guidelines for the Care and Use of Animals for Scientific Purposes at Tokai University and approved by the Institutional Review Board of Tokai University School of Medicine (Permit No. 162043).

Female C57BL/6 mice (obtained from CREA, Japan) were housed under a 12-hour light/dark cycle in a temperature and humidity-controlled environment (22~24℃, 55% relative humidity) with free access to food and water. Animals were randomly allocated to the control or AM-administered groups.

Administration of AM

AM powder (Tochimoto, Japan) was mixed in sterilized 0.5% methyl cellulose 400 (w/v) (Wako, Japan) and administered to mice by gavage once, 1 hour before the experiment, at a dose of 0.5 g/kg body weight/day. An equal volume of 0.5% methyl cellulose 400 was administered to the mice on the same schedule as the control (vehicle).

Isoflurane Anesthesia

Anesthesia was induced with 5% isoflurane (in the air) in an induction chamber. Animals were then rapidly switched from the induction chamber to a nose cone attached to a stereotaxic frame where the animals were allowed to freely breathe isoflurane (2.5%) for maintenance. The depth of anesthesia was frequently assessed (5~10 min intervals) with the heart rate and breathing pattern.

Renal Ischemia-Reperfusion

Transient renal ischemia was induced using a recently described technique (Boesen, 2016; Le Clef, Verhulst, D'Haese, Vervaet, 2016). In advance, about 0.2 ml of blood sample from each mouse was collected longer than 2 hours before surgery. Anesthetized mice were surgically operated on at 37.0℃ using a heating pad. The left renal artery was exposed and occluded by non-traumatic small clips for 30 minutes. After occlusion, the clips were carefully removed, and the artery was inspected for reperfusion. After 2 hours reperfusion, the mice were maintained temporarily in a case at 30℃. Sham-operated animals were subjected to the same anesthetic and surgical treatments. Twenty hours after the unilateral renal IR, about 0.2 ml of blood sample was collected from each mouse. The blood samples before and after renal IR were incubated for longer than 2 hours, centrifuged for 10 minutes, and each serum was collected. These serum samples were measured for the inspection items associated with kidney function, such as blood urea nitrogen (BUN) and serum creatinine (SCr) (measured in Oriental Yeast Co., Ltd, Japan).

Statistics

Statistical analysis was carried out with Graph Pad Prism 6 (La Jolla, CA, USA). Comparison of biochemical data between the sham-operation and unilateral IR samples was performed using Kruskal-Wallis test. Data are presented as mean ± standard error of the mean. $P < 0.05$ was considered significant.

RESULTS

AM effects in mice with moderate AKI

We first tried to establish a mouse model suitable for investigation of AM effects against AKI. We preliminarily used unilateral renal IR with a short duration of ischemia (30 minutes) as moderate AKI (Fig. 1). BUN levels were slightly but significantly increased 20 hours after IR (Fig. 2A, solid arrowhead); however, levels of another serum parameter associated with the renal dysfunction were not significant (data not shown). We hypothesized that the normalizing effects of AM would be universal and independent; therefore, IR-induced renal dysfunction would likely be effective not only in younger but also in older mice. We therefore used various aged mice to examine the universal mechanism of AM. As expected, AM administration attenuated the IR-induced BUN increase; however, the declined changes were not significant and showed a statistically large variance (Fig. 2A, open arrowhead). We did further statistical research and identified a significant difference. Unexpectedly, AM prevents an IR-induced BUN increase in mice older than 40 weeks (Fig. 2B) indicating that AM is effective for AKI in an age-dependent manner. For elderly people, AM administration could be

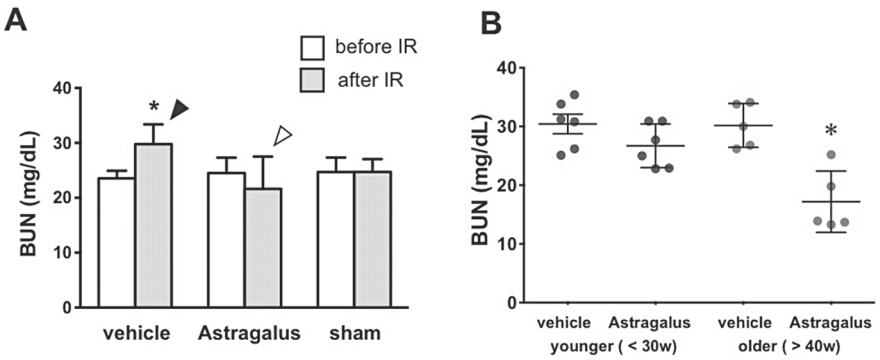

Figure 2. A. BUN is significantly elevated in 20 hours after retinal IR using mice of various ages (open arrowhead). AM controlled the elevated BUN after renal IR (solid arrowhead). Normalized BUN levels showed no significant difference after IR in the vehicle because of increased variation. All values are shown as the mean ± SEM (n = 12). *P < 0.05 for BUN compared with before IR in the vehicle.

B. AM-normalized BUN showed an age-dependent manner. The mice were categorized in either the lower or older age groups. An AM-normalizing effect of BUN was observed in the older group. All values are shown as the mean ± SEM (n = 6). *P < 0.05 for BUN compared with older age in the vehicle.

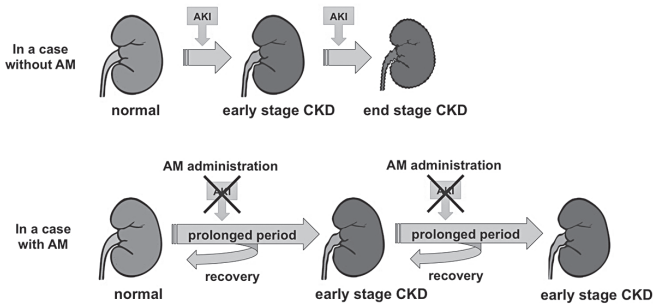

Figure 3. Schematic representation of a potent AM mechanism for CKD therapy. To prolong the progress of CKD, AM might remove moderate AKI causing higher levels of BUN but not SCr.

an attractive measure to improve the slight renal dysfunction caused by AKI.

DISCUSSION

We examined the pharmacological effects of AM using mice with unilateral renal IR as an AKI model. The IR condition caused elevated BUN levels; however, the difference was insignificant (Fig. 2A), compared to other reports using unilateral IR (Boesen, 2016; Le Clef et al., 2016). Similar data has been defined in mice with pre-renal AKI, also called acute renal failure, showing a slight increase of BUN probably resulting from pre-renal azotemia and mild renal tubular damage previously undetectable (Doi, Katagiri, Negishi, Hasegawa, Hamasaki, Fujita, Matsubara, Ishii, Yahagi, Sugaya, Noiri, 2012). Recently, it is widely supported that AKI plays a possible role not only in the early stage of the pathogenesis of CKD but also in its progression (O'Sullivan, Hughes, Ferenbach, 2017; Rifkin *et al*., 2012). Furthermore, even if AKI-induced dysfunction could be recovered clinically, it is obvious that the progression to CKD remains lasting (Murugan *et al*., 2011). Therefore, we strongly suggest that mild and day-to-day emerged AKI is a critical factor for the pathogenesis and development of CKD. In this study, we used a mouse model for moderate AKI and showed that AM normalized the slight reduction of renal function (Fig. 2). Actually, evidence has shown an effectiveness of different herbs to treat renal problems (Boozari, Hosseinzadeh, 2017); however, our findings suggests AM could be used in the treatment of minor physical problems resulting in initial and/or developing factors for severe renal diseases. Therefore, it is likely that AM targets the moderate AKI showing few clinical observations but definitely causing CKD. Currently accumulated evidence has been already provided that AM could be an option to treat IR-induced injury and dysfunction in the cerebrum, heart, and small intestine; therefore, AM could be a possible choice to prevent IR injury (Cai *et al*., 2001; Han *et al*., 2017; Huang *et al*., 2015a; Huang *et al*., 2015b; Li *et al*., 2017a; Song *et al*., 2009; Song *et al*., 2008). Compared to evidence from other organs, only few findings about the AM effects against renal injury and damage were demonstrated (Shahzad, Shabbir, Wojcikowski, Wohlmuth, Gobe, 2016; Song *et al*., 2009; Song *et al*., 2008). Considering the evidence, we suggest that AM effects show an unstable manner in severe renal conditions.

Oral administration of AM could normalize the elevated renal dysfunction after IR (Fig. 2A); however, the changed levels did not show a significant difference compared to those without AM, i.e., vehicle and sham (Fig. 2A). Further statistical examination revealed that AM had an age-dependent effect against IR-induced renal dysfunction (Fig. 2B). Recent studies demonstrated that the kidney after IR showed activities for another pathway leading to the recovery processes, which was organized by functional molecules, including cytokines, growth factors, and peptides, e.g., endothelins (Boddu *et al*., 2017; Boesen, 2016; Le Clef *et al*., 2016; Li *et al*., 2017b; Vincent, Okusa, 2014). AM causes activation of these molecules in AKI and contributes to regeneration and regrowth of tubules, inflammatory inhibition, and directly preventing CKD (Song *et al*., 2008). Furthermore, recent studies also showed that these recovery activities depended on aging and sex (Boddu *et al*., 2017; Li *et al*., 2017b; Meersch, Schmidt, Zarbock, 2017; Vincent *et al*., 2014). Expectedly, AM effects, as shown in Fig. 2B, could be significantly recovered in older mice, suggesting that the endogenous renal recovery activities, normally down-regulated by aging, could be activated by AM administration. On the other hand, AM-induced renal recovery in younger mice was not successful (Fig. 2B). AM exhibits multiple effects involved in antioxidative recovery (Song *et al*., 2008) and antitumorigenetic apoptosis (Auyeung *et al*., 2016; Fu *et al*., 2014), both of which seem opposite but might depend on various organ situations. Taken together, we suggest that in older kidney, even a moderate risk factor can cause an emergency, and AM effects alleviate the condition. AM effects in younger kidney might be inferior to age-dependent endogenous recovery. Also, our experimental moderate AKI, inducing only pre-renal azotemia, might be an insignificant factor in younger kidney. On the other hand, the characterization of the age-dependent AM effects warrant further investigation; however, we cannot exclude the possibility that AM possess an activity compatible to age-upregulated function in the treatment of IR.

CONCLUSION

AM administration can protect the kidney against moderate IR injury, which would play a critical role in older kidney. Because AM normalized renal dysfunction in older mice, this effect showed an age-dependent manner. These findings indicate that AM administration, at least in part, can reduce day-to-day generated AKI, which is ineffective to younger kidney, progressing to future emergency conditions (Fig. 3).

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