Ginsenoside Rg1 and astaxanthin act on the hypothalamus to protect female mice against reproductive aging

Xiaoman Dai[1,2], Guirong Zeng[2], Luyan Hong[2], Qinyong Ye[1,2], Xiaochun Chen[1,2], Jing Zhang[1,2]

[1]Department of Neurology and Geriatrics, Fujian Institute of Geriatrics, Fujian Medical University Union Hospital, Fuzhou, Fujian 350001, China;
[2]Fujian Key Laboratory of Molecular Neurology, Institute of Neuroscience, Fujian Medical University, Fuzhou, Fujian 350005, China.

To the Editor: Female infertility due to reproductive aging has become a prominent concern in the population. Evidence indicates that oxidative stress is one of the dominant mechanisms underlying reproductive aging.[1,2] Although female reproductive aging involves gradual changes to the hypothalamic-pituitary-ovarian (HPO) axis, previous studies support a key involvement of the hypothalamus.[3,4] In our recent dataset, it was shown that estrogen-induced oxidative stress led to the senescence of hypothalamic astrocytes that predated reproductive dysfunction.[3] Growing evidence has demonstrated that antioxidants might delay reproductive aging by reducing oxidative damage to ovaries.[1,2] However, the effect of antioxidants on the hypothalamus has not been reported. Astaxanthin (AST), 3,3′-dihydroxy-β-carotene-4,4′-dione, is a xanthophyll carotenoid found predominantly in marine animals such as salmon, trout, lobster, and shrimp. Ginseng is a tonic drug in traditional Chinese medicine. Ginsenoside Rg1, a class of steroidal glycosides, is a prominent active anti-aging ingredient within ginseng responsible for these effects. Notably, the two antioxidants freely cross the blood-brain barrier. In this study, we evaluated the effects of these two natural compounds on the hypothalamus and ovaries in protecting female mice from reproductive aging.

Intact naturally-aging mice were used to study the functional mechanisms of reproductive decline. Young mice were 3 to 4 months old and middle-aged mice were 9 to 10 months old in this study. Vaginal smears were used to monitor the estrous cycle daily, which were divided into diestrus, proestrus, estrus, and metestrus according to the cell type(s) present [Supplementary Figure 1A, http://links.lww.com/CM9/A579]. The average estrous cycle of young mice was 4 to 5 days, while the middle-aged mice showed irregular estrus cycles, which was more than 6 days on average [Supplementary Figure 1B, http://links.lww.com/CM9/A579]. We then explored the age-related changes to the HPO axis, which is the basis for the regular estrous cycles. In the present study, the senescence β-galactosidase staining (SA-β-Gal) combined with glial fibrillary acidic protein immunohistochemistry showed higher SA-β-Gal activity in the hypothalamic astrocytes of the middle-aged mice than in that of the young mice [Supplementary Figure 1C and 1E, http://links.lww.com/CM9/A579]. Consistent with this result, increased peroxidase activity was found in the middle-aged mice [Supplementary Figure 1D and 1F, http://links.lww.com/CM9/A579]. The ovarian morphology of the young and middle-aged groups was observed by paraffin section and hematoxylin/eosin staining [Supplementary Figure 1G, http://links.lww.com/CM9/A579]. Follicles and corpus luteum at different stages of development were observed in both the young- and middle-aged groups, although mice showed a reduced number of primary follicles, secondary follicles, and antral follicles [Supplementary Figure 1G and 1H, http://links.lww.com/CM9/A579]. As luteinizing hormone (LH) levels can indirectly reflect the functionality of the pituitary gland, we quantified LH via enzyme-linked immunosorbent assay. No significant difference was found between the middle-aged and the young mice [Supplementary Figure 1I, http://links.lww.com/CM9/A579]. The aging-related changes to the hypothalamus and ovary might prove to be pivotal in initiating irregular estrous cycles in the mid-aged mice.

Our group and others have shown that the oxidative stress in the hypothalamus and ovaries contributes to aging-related reproductive function decline in female mice.[1-3] Rg1 and AST have numerous pharmacological effects relevant to anti-oxidation and anti-aging, so we detected the effects of the two antioxidants on reproductive function. A total of 80 young mice with regular estrous cycles were selected and divided into four groups: a saline control group, an Rg1 group, an olive oil control group, and an AST group. As astrocytes show signs of cellular senescence at an early age of 3 months, drug intervention

Access this article online

Quick Response Code:

Website: www.cmj.org

DOI: 10.1097/CM9.0000000000001542

Correspondence to: Jing Zhang, Department of Neurology and Geriatrics, Fujian Institute of Geriatrics, Fujian Medical University Union Hospital, 29 Xinquan Road, Fuzhou, Fujian 350001, China
E-Mail: drzj@163.com

Copyright © 2021 The Chinese Medical Association, produced by Wolters Kluwer, Inc. under the CC-BY-NC-ND license. This is an open access article distributed under the terms of the Creative Commons Attribution-Non Commercial-No Derivatives License 4.0 (CCBY-NC-ND), where it is permissible to download and share the work provided it is properly cited. The work cannot be changed in any way or used commercially without permission from the journal.

Chinese Medical Journal 2022;135(1)
Received: 09-11-2020; **Online:** 13-05-2021 **Edited by:** Lishao Guo

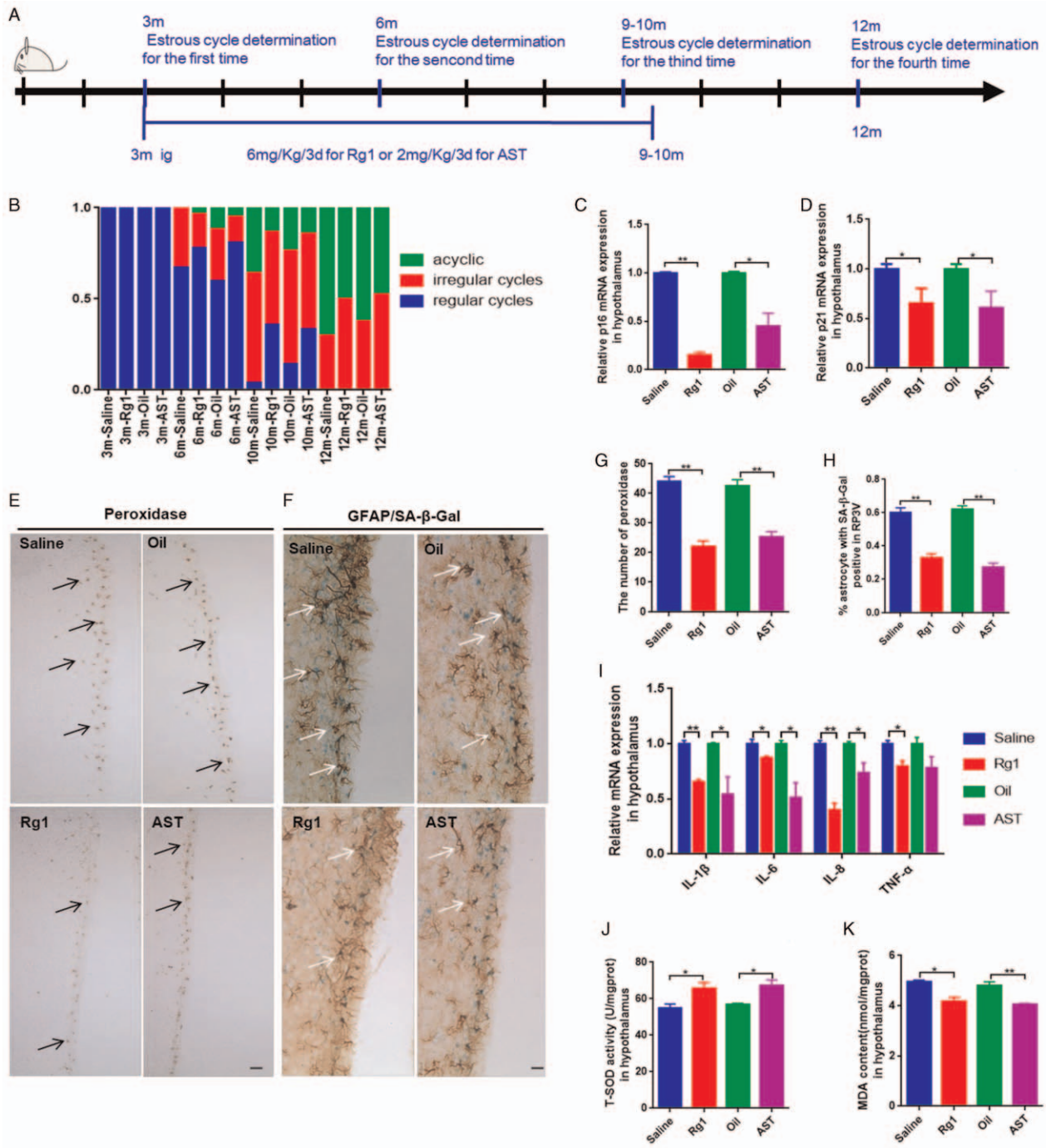

Figure 1: The effect of chronic Rg1 and AST administration on the estrous cycle and hypothalamic senescence associated secretory phenotype (SASP). (A) Experimental setup used for assessing the effects of chronic Rg1 and AST administration on the estrous cycles of the C57BL/6J female mice. Rg1 (6 mg/kg per 3 days) was injected intraperitoneally every three days for 6 months. AST (2 mg/kg per 3 days) was injected intraperitoneally every three days for 6 months. (B) The percentages of regular estrous cycle, irregular estrous cycle, and continuous diestrus or continuous estrus (acyclic). (C and D) Effects of Rg1 and AST on the mRNA levels of *p16* and *p21* in the hypothalamus as determined by qPCR. $n = 3$. (E) Peroxidase staining in the hypothalamus, black arrows representing peroxidase. Scale bar = 200 μm. (F) Dual-label immunohistochemistry of astrocytes by glial fibrillary acidic protein staining (brown) and by SA-β-Gal staining (blue) in the Rg1, saline, AST, and oil group mice ($n = 5$), white arrows representing SA-β-Gal positive astrocytes. Scale bar = 200 μm. (G) Quantification of peroxidase-positive astrocytes. (H) Quantification of SA-β-Gal positive astrocytes. (I) Effects of Rg1 and AST on the levels of *IL-1β*, *IL-6*, *IL-8*, and *TNF-α* mRNA in hypothalamus, as determined by qPCR. $n = 3$. (J) T-SOD activity in the hypothalamic was evaluated by chemical colorimetric analysis. $n = 3$. (K) MDA contents in the hypothalamic were evaluated by chemical colorimetric analysis. $n = 3$. Data are presented as mean ± standard error of the mean. $^{*}P < 0.05$, $^{**}P < 0.01$, as compared with the control group. AST: Astaxanthin; IL-1β: Interleukin-1β; mRNA: Messenger RNA; qPCR: Quantitative polymerase chain reaction; Rg1: Ginsenoside; SA-β-Gal: Senescence β-galactosidase staining; TNF-α: Tumor necrosis factor-α.

started at 3 months of age and was maintained until 9 to 10 months age. The measurement of the second and third estrous cycles was conducted at 6 and 10 months of age, respectively. The fourth estrous cycle was measured at 12 months, as shown in Figure 1A. With advancing age, the percentage of mice with regular estrous cycles decreased in the saline group, while Rg1 treatment increased the percentage of mice with regular estrous cycles in comparison [Figure 1B]. In the AST treatment group, the percentage of mice with regular estrous cycles

was higher than that of the olive oil group at 6 and 10 months. Unfortunately, no difference in the percentage of mice with regular estrous cycles was found among all the groups at 12 months. The findings suggest that chronic Rg1 and AST administration delay the irregularity of the estrous cycle in female mice during aging.

In order to illuminate how Rg1 and AST delay reproductive aging, we first examined the effect of Rg1 and AST on the senescence status of the hypothalamus in middle-aged mice. Senescence associated secretory phenotype (SASP) is characterized by an up-regulation of *p16* and *p21*, positive staining for SA-β-Gal, and secretion of proinflammatory cytokines. After testing for these metrics, our data showed that Rg1 treatment reduced the gene expression levels of *p16* and *p21* in the hypothalamus of middle-aged mice ($F = 6.528$, $P < 0.01$ for *p16*; $F = 9.013$, $P < 0.05$ for *p21*) [Figure 1C and 1D]; AST treatment decreased the expression of *p16* and *p21* in the hypothalamus compared to the oil group ($F = 57.37$, $P < 0.05$ for *p16*; $F = 10.92$, $P < 0.05$ for *p21*) [Figure 1C and 1D]. Immunohistochemistry results showed that Rg1 and AST treatments were associated with decreased peroxidase activity when compared to the control group ($F = 2.189$, $P < 0.01$ for Rg1; $F = 1.087$, $P < 0.01$ for AST) [Figure 1E and 1G]. Similarly, Rg1 and AST treatments reduced the number of the astrocytes with SA-β-Gal staining ($F = 1.144$, $P < 0.01$ for Rg1; $F = 1.657$, $P < 0.01$ for AST) [Figure 1F and 1H]. And then, the expression levels of interleukin-1β (*IL-1β*), *IL-6*, *IL-8*, and tumor necrosis factor-α (*TNF-α*) were significantly decreased in the hypothalamus of the Rg1 group as compared to the saline control group ($F = 1.316$, $P < 0.01$ for *IL-1β*; $F = 7.737$, $P < 0.05$ for *IL-6*; $F = 4.68$, $P < 0.01$ for *IL-8*; $F = 3.583$, $P < 0.05$ for *TNF-α*) [Figure 1I]. Meanwhile, the levels of the proinflammatory cytokines were notably reduced in the AST treatment group when compared to the olive oil control group ($F = 186.3$, $P < 0.05$ for *IL-1β*; $F = 21.37$, $P < 0.05$ for *IL-6*; $F = 20.79$, $P < 0.05$ for *IL-8*) [Figure 1I].

To further explore the possible mechanism of antioxidants (Rg1 and AST) in alleviating reproductive aging, we detected the redox status in the hypothalamus upon Rg1 and AST treatment. Total superoxide dismutase (T-SOD) is a critical antioxidative enzyme that alleviates oxidative stress produced during aging. Malondialdehyde (MDA) is widely used as an oxidative stress biomarker. Thus, we evaluated T-SOD activity and MDA content in the hypothalamus. Compared to the saline group, T-SOD activity was significantly increased and MDA levels were reduced in the hypothalamus of the Rg1 group ($F = 1.646$, $P < 0.05$ for T-SOD; $F = 5.226$, $P < 0.05$ for MDA) [Figure 1J and 1K]. Moreover, AST treatment also led to an increase of T-SOD activity significantly and reduced the MDA level ($F = 11.71$, $P < 0.05$ for T-SOD; $F = 12.43$, $P < 0.01$ for MDA) [Figure 1J and 1K]. These data suggest that long-term Rg1 and AST treatment down-regulate the hypothalamic SASP through antioxidant effects. Of note, this is the first time that antioxidants have been documented to delay reproduction senescence in hypothalamic tissues.

Similarly, we tested the effects of long-term administration of Rg1 and AST on ovaries. Firstly, neither AST nor Rg1 affected the number of growing or mature follicles compared with the control group [Supplementary Figure 2A and 2B, http://links.lww.com/CM9/A579]. Moreover, the Rg1 and AST treatments had no significant effect on the uterine and ovarian weight compared to the control groups, respectively [Supplementary Figure 2C and 2D, http://links.lww.com/CM9/A579]. Then, we found that Rg1 and AST treatment decreased the expression of *p16, p21, IL-1β*, *IL-6*, *IL-8*, and *TNF-α* in ovaries, compared to the respective control groups [Supplementary Figure 2E–J, http://links.lww.com/CM9/A579]. Finally, T-SOD activity was significantly increased and the MDA level was remarkably declined in the ovaries of the Rg1 or AST group [Supplementary Figure 2K and 2L, http://links.lww.com/CM9/A579]. These data suggest that long-term Rg1 and AST treatment down-regulate ovarian SASP through antioxidant effects.

Taken together, the results of this study suggest that chronic administration of Rg1 and AST can effectively enhance fertility outcomes, which may in part involve distinct antioxidative effects on the ovary and hypothalamus of naturally aging mice. However, the specific targets and molecular mechanisms of antioxidants need to be further studied, so as to provide new targets for more effective treatment of reproductive aging.

Funding

The study was supported by grants from the National Natural Science Foundation of China (No. 81671400) and the Fujian Province Health and Family Planning Youth Research Project (No. 2018-1-45).

Conflicts of interest

None.

References

1. Wang S, Zheng Y, Li J, Yu Y, Zhang W, Song M, *et al*. Single-cell transcriptomic atlas of primate ovarian aging. Cell 2020;180:585–600.e19. doi: 10.1016/j.cell.2020.01.009.
2. Peters AE, Mihalas BP, Bromfield EG, Roman SD, Nixon B, Sutherland JM. Autophagy in female fertility: a role in oxidative stress and aging. Antioxid Redox Signal 2020;32:550–568. doi: 10.1089/ars.2019.7986.
3. Dai X, Hong L, Shen H, Du Q, Ye Q, Chen X, *et al*. Estradiol-induced senescence of hypothalamic astrocytes contributes to aging-related reproductive function declines in female mice. Aging (Albany NY) 2020;12:6089–6108. doi: 10.18632/aging.103008.
4. Zhang J, Yang L, Lin N, Pan X, Zhu Y, Chen X. Aging-related changes in RP3V kisspeptin neurons predate the reduced activation of GnRH neurons during the early reproductive decline in female mice. Neurobiol Aging 2014;35:655–668. doi: 10.1016/j.neurobiolaging.2013.08.038.

How to cite this article: Dai X, Zeng G, Hong L, Ye Q, Chen X, Zhang J. Ginsenoside Rg1 and astaxanthin act on the hypothalamus to protect female mice against reproductive aging. Chin Med J 2022;135:107–109. doi: 10.1097/CM9.0000000000001542