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Anti-aging effect of astragalosides and its mechanism of action

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ABSTRACT

AIM: To study the anti-aging effect of astragalosides (AST) and its mechanism of action. **METHODS:** Rotating rod test and step-down type passive avoidance test were performed to determine the effects of AST on motor and memory of *D*-galactose (*D*-gal)-induced senescent mice and the middle-aged mice. The proliferative response of splenocytes induced by Con A or LPS, IL-2 production of splenocytes induced by ConA of *D*-gal-treated mice and the middle-aged mice were also measured. **RESULTS:** AST (40 mg· kg^{-1}· d^{-1}, ig, for 10 weeks) was found to ameliorate age-related alternations in both motor response and memory, enhance the deteriorated cellular immunity in *D*-gal-treated mice and the pre-aged (17-month-old) mice. **CONCLUSION:** AST has an anti-aging effect on *D*-gal-induced senescent mice and has the effect of delaying senility of the middle-aged mice, which was related to its improvement of brain function and immunomodulatory effects.

INTRODUCTION

Aging is a natural phenomenon that has a common symptom —motor and memory dysfunction. Also, there are a lot of assumptions concerning senescence, such as free radical degeneration and immune system dysfunction.

Astragalus is a kind of Chinese tonic herbs. Its active part is astragalosides (AST) and polysaccharides which are extracted from the root of *Astragalus membranceus* (Fisch) Bge[1,2]. Previous studies from our laboratory showed that AST possessed an anti-aging effect, probably being related to its anti-oxidative properties[3]. A pilot study from our laboratory demonstrated that AST had an immunomodulatory effect. However, the relationship of anti-aging effect of AST to immune response is unknown. The present study was therefore designed to investigate the anti-aging effect of AST and its relation with immunity, exploiting *D*-gal-induced senescent mice and the middle-aged mice.

MATERIALS AND METHODS

Mice Kunming strain mice [male, young: 3-month-old, weighing (30±3) g; middle age, 14-month-old, weighing (55±4) g] and C57BL/6J mice [6-8 weeks old, weighing (20±3) g] were supplied by Experimental Animal Center of Anhui Medical University (Grade II, Certificate No 01).

Materials Astragalosides (AST) was provided by Jiangsu Institute of Materia Medica (Nanjing, China) and dissolved in 1 % sodium carboxymethylcellulose (CMC-Na). The content of AST was 100 %. Vitamin E (VE, Merck) was dissolved in 1 % CMC-Na with

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absolute ethanol (less than 0.2 %, v/v). *D*-galactose (*D*-gal, Sigma) was dissolved in 0.9 % sterile saline at a concentration of 0.5 % and stored at 4 °C.

Concanavalin A (ConA) and lipopolysaccharides (LPS) were obtained from Sigma; [^{3}H]Thymidine (TdR, specific radioactivity 666 TBq/mol) was obtained from Chinese Academy of Atomic Energy Science, Beijing; RPMI-1640 medium was obtained from Gibco which was supplemented with HEPES buffer 25 mmol/L, sodium pyruvate 1 mmol/L, *L*-glutamine 2 mmol/L, 2-mercaptoethanol 50 μmol/L, penicillin 100 kU/L, streptomycin 100 mg/L and 10 % new born bovine serum and were adjusted to pH 7.2; Bovine serum was supplied by Department of Microbiology, Anhui Medical University.

Induction of senescence Injections of *D*-galactose were performed as previously described[4]. *D*-gal (40 mg/kg) was injected subcutaneously every other day for 10 weeks. Mice were orally given drugs or dissolvant once a day for 10 weeks. Middle-aged mice were also orally given drugs or dissolvant once a day for 12 weeks.

Rotating-rod test The rota-rod apparatus consisted of 1 base, 2 poles, and 1 rod. The rod is 60 cm long, 25 cm away from the base supported by 2 poles. At the beginning of test, mice were put into the rod slightly. Then the rod was revolved by hand at the rotating speed of 10 r/min for 1 min. The falling number of mice was recorded and the falling percent was calculated.

Step-down type passive avoidance test The apparatus consisted of an acrylic box with a stainless-steel grid floor. A wooden platform was fixed in the center of the box. Electric shocks (40 mV) were delivered to the grid floor for 3 s with an isolated pulse stimulator. At the beginning of training, mice were placed in the box to adapt for 3 min. Then mice were put onto the platform slightly. When the mice stepped down and put all its paws onto the grid floor, it would jump to the platform as shock happened to be delivered.

Step-down latency (time of staying on the platform, SDL) and the number of errors (NOE) was recorded within 5 min and repeated 24 h after training.

Proliferative response of splenocytes The splenocyte suspension (1×10^{10}/L) was prepared in a general way. Splenocyte suspension 100 μL was seeded on 96-well microtiter plate in the presence of ConA (final concentration 3 mg/L) or LPS (final concentration 6 mg/L). The splenocytes were incubated at 37 °C in 5 % CO_2 incubator for 48 h and pulsed with [^{3}H]TdR 20 μL (1.4×10^4Bq per well) 6 h before harvesting. The cells were harvested onto type-69 glass fiber filters. The radioactivity was measured with liquid scintillation counter. The results were expressed as means of Bq of triplicate wells.

IL-2 assay Splenocytes suspension (1×10^{10}/L) was prepared in a general way. Splenocytes suspension 500 μL was seeded on 24-well culture plate in the presence of ConA 500 μL (final concentration 3 mg/L). The splenocytes were incubated at 37 °C in an atmosphere of 5 % CO_2 and 95 % air for 48 h. Then the plate was centrifuged (2690×*g*, 10 min) at 4 °C. The supernatant contained IL-2 was collected and stored at -20 °C until tested for IL-2 activity. Mouse splenocytes activated by ConA were prepared by described method[5] that was used for measuring IL-2 activity. The supernatant containing IL-2 was diluted by 40 times. Activated splenocyte suspension (2×10^9/L) 100 μL was seeded on 96-well microtiter plate in the presence of the dilution 100 μL . The splenocytes were incubated at 37 °C in a 5 % CO_2 incubator for 24 h and pulsed with [^{3}H]TdR 20 μL 6 h before harvesting. The cells were harvested onto type-69 glass fiber filters. The radioactivity was measured with liquid scintillation counter. The results were expressed as the means of Bq of triplicate wells.

Statistical analysis Data were expressed as mean±SD and compared by the Student's *t*-test or χ^2 test.

RESULTS

Effects of AST on the behavior of *D*-gal-treated mice *D*-gal (40 mg/kg, sc every other day for 10 weeks) increased the falling rate of mice determined by rotating-rod test ($P<0.05$), shortened SDL ($P<0.01$) and increased NOE ($P<0.05$) determined by step-down test. AST (40 mg/kg, ig once a day for 10 weeks) and VE (50 mg/kg) markedly improved motor and memory dysfunction of *D*-gal-treated mice (Tab 1).

Effects of AST on the immune function of *D*-gal-treated mice In *D*-gal-treated mice, the thymus index, the proliferative response and IL-2 production of splenocytes induced by ConA markedly decreased; while the spleen index and the proliferative response of B cells induced by LPS did not change markedly ($P>0.05$). AST and VE improved the low cellular immunity to some degree (Tab 2).

Tab1. Effect of AST on the behavior of *D*-galactose-treated mice. n=10. Mean±SD. ^{b}P<0.05, ^{c}P<0.01 *vs* normal control. ^{e}P<0.05, ^{f}P<0.01 *vs* *D*-gal control.

Group	Dose /mg·kg^{-1}	Motor coordination Fall number	Fall rate /%	Memory SDL/s	NOE
Normal	-	2	20	89±13	1.0±0.7
D-gal	-	7^{b}	70	44±17^{c}	1.9±0.9^{b}
D-gal+AST	40	2^{e}	20	84±12^{f}	0.9±0.6^{f}
D-gal+VE	50	2^{e}	20	81±14^{f}	0.9±0.6^{f}

Tab 2. Effect of AST on indices of thymus and spleen, ConA-induced proliferative response, and IL-2 synthesis by splenocytes from *D*-gal-treated mice. Mean±SD. ^{b}P<0.05, ^{c}P<0.01 *vs* normal control. ^{e}P<0.05, ^{f}P<0.01 *vs* *D*-gal control.

Group	Dose/ mg·kg^{-1}	Indices/mg.g^{-1} BW (n=10) Thymus	Spleen	Proliferation/ Bq (n=4)	IL-2 activity/ Bq (n=4)
Normal	-	1.11±0.20	2.6±0.8	933±100	130±33
D-gal	-	0.81±0.25^{c}	2.3±0.6	280±93^{c}	53±20^{b}
D-gal+AST	40	1.07±0.15^{f}	2.7±0.7	700±133^{bf}	110±37^{e}
D-gal+VE	50	1.01±0.12^{f}	2.3±0.6	700±200^{f}	130±37^{e}

Effects of AST on the behavior of middle-aged mice In 17-month-old mice, the falling rate increased (P<0.05); SDL shortened and NOE increased (P<0.01).

AST (40 mg/kg, ig once a day for 12 weeks) and VE (50 mg/kg) lowered the falling rate (P<0.05); AST lowered the NOE of 17-month-old mice (P<0.05) and had the tendency of prolonging SDL; VE prolonged SDL (P<0.01) and had the tendency of reducing NOE (Tab 3). This suggests that AST and VE improved motor and memory dysfunction of the pre-aged (17-month-old) mice.

Tab 3. Effect of AST on the behavior of the pre-aged (17-month-old) mice. n=10. Mean±SD. ^{b}P<0.05, ^{c}P<0.01 *vs* control 6 month. ^{e}P<0.05, ^{f}P<0.01 *vs* control 17 month.

Group	Dose /mg·kg^{-1}	Motor coordination Fall number	Fall rate /%	Memory SDL/s	NOE
Control					
6 month	-	1	10.0	73±10	0.9±0.6
17 month	-	7^{b}	70.0	31±7^{c}	2.5±1.0^{c}
17 mon+AST	40	2^{c}	20.0	39±12^{c}	1.7±0.7^{ce}
17 mon+VE	50	1^{e}	10.0	46±7^{cf}	1.5±0.5^{b}

Effects of AST on immune function of middle-aged mice The thymus index, the proliferative response and IL-2 production of splenocytes induced by ConA of 17-month-old mice markedly decreased; while the spleen index did not change (P>0.05). AST and VE improved the low cellular immunity of the pre-aged (17-month-old) mice to some degree (Tab 4).

DISCUSSION

The motor function and memory would decline with age. This might be related to the declining of advanced function of the brain[6]. In this study, step-down type avoidance and rotating rod tests were used to examine the long-term memory and motor function, which are as index of senescence of mice.

Tab 4. Effect of AST on indices of thymus and spleen, ConA-induced proliferative response, and IL-2 synthesis by splenocytes from the pre-aged (17-month-old) mice. Mean±SD. ^{b}P<0.05, ^{c}P<0.01 *vs* control 6 month. ^{e}P<0.05, ^{f}P<0.01 *vs* control 17 month.

Group	Dose /mg·kg^{-1}×12 weeks	Indices/mg.g^{-1} BW (n=10) Thymus	Spleen	Proliferation /Bq (n=4)	IL-2 activity /Bq (n=4)
Control					
6 month	-	3.4±0.8	61±15	667±167	150±20
17 month	-	0.25±0.07^{c}	58±15	223±10^{c}	100±13^{b}
17 month+AST	40	0.55±0.10^{cf}	55±6	833±233^{f}	120±10^{be}
17 month+VE	50	0.34±0.09^{c}	60±11	480±97^{f}	150±13^{f}

There are several assumptions concerning aging, of which the well-known theories are free radical theory and immune theory. The free radical theory states that oxygen free radicals are the important factor involved in the phenomenon of biological aging[7]. The immune theory states that the decrease of immune function, especially cellular immune function is the main reason of aging[8], in which the most obvious change is the decrease of interleukine-2 (IL-2) production and IL-2 receptor expression[9]. Previous studies showed that chronic injections of *D*-gal subcutaneously into mice induced changes which resembled accelerated aging. The aging model shows neurological impairment, decreased activity of anti-oxidant enzymes, and poor immune responses[10]. Therefore, we exploited *D*-gal-treated mice as experimental senile model to investigate the anti-aging effect of AST as well as the middle-aged (14-month-old) mice.

Our previous study demonstrated that AST had anti-oxidative and immunomodulatory properties (unpublished data). Treatment with AST (40 mg·kg^{-1}·d^{-1}, ig) for 10 weeks would lower the content of MDA and restore activities of MnSOD, GSHpx and GSH/GSSG ratio in mitochondria of *D*-gal-treated mice. Treatment with AST (40 mg·kg^{-1}·d^{-1}, ig) for 3 months had the same effect on 17-month-old mice. AST has an anti-aging effect, probably being related to its anti-oxidative properties[3]. Therefore, in this study, we investigate the relationship of the immunomodulatory effect of AST, especially its effect on cellular immunity and IL-2 production, to its anti-aging effect. As to the dose of this study, it was designed according to reference[11] and the results of our previous studies of AST. The dose rate of pharmacodynamic study *in vivo* is often 1:2:4 or 1:3:9[11]. In previous studies we adopted the dose of 20, 40, and 80 mg/kg or 10, 30, and 90 mg/kg to investigate its anti-inflammatory, immunomodulatory, and anti-oxidative effects. The results of many studies showed that AST was ineffective at 10, 20, and 30 mg/kg, while it was effective at 40, 80, and 90 mg/kg[3,12]. A pilot study from our laboratory demonstrated that AST had an anti-aging effect at 40 and 80 mg/kg, while it was ineffective at 20 mg/kg. Therefore, we adopted 40 mg/kg as the dose of the present study.

The results of our study showed that the functions of motor and memory, the thymus index, the proliferative response and IL-2 production of splenocytes induced by ConA of *D*-gal-treated mice and the pre-aged (17-month-old) mice were all lower than those of the young mice. Treatment with AST could restore the functions of motor and memory; enhance the thymus index and cellular immunity of *D*-gal-treated mice and the pre-aged mice. This suggested that AST has an anti-aging effect on *D*-gal-treated mice and has the effect of delaying in senility of middle-aged mice. VE also has the same effects.

Our previous study has demonstrated that AST has a dual immunomodulatory effect. But AST has no effect on the immune function of normal animals (unpublished data). This suggests that the immunological enhancement effect of AST on aged mice is a tonic effect.

In conclusion, AST has an anti-aging effect on *D*-gal-treated mice and has the effect of delaying in senility of middle-aged mice, which was related to its immunomodulatory effect.

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