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Original article

Age Supplementation Modulates IL-6, IL-10 and HSP27 Response in Healthy Men: A pilot study

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Abstract. *Background:* Aged garlic extract (AGE) is a dietary supplement reported to have health benefits such as protection against oxidative stress, inflammatory disorders, and dyslipidemia. The aim of the study to investigate effect of the short term AGE supplementation on the serum cytokines (IL-6 and IL-10) and biochemical parameters (HSP27, TAS, TOS, cortisol and testosterone) in healthy men. *Methods:* In this study the effects of AGE on the serum levels of interleukin-6 (IL-6), interleukin-10 (IL-10), heat shock protein 27 (HSP27), testosterone, cortisol, total antioxidant status (TAS), and total oxidant status (TOS) in healthy subjects was investigated. Sedentary healthy men (n=6) participated in this study and ingested AGE (5 ml) for 10 days. During the study blood samples were taken in the morning before and 10 days after AGE supplementation, followed by another sample 10 days post-supplementation (wash out) period. The serum was analysed for biochemical parameters IL-6, IL-10, HSP27, cortisol, testosterone, TAS and TOS. These parameters were evaluated at the beginning of the study, end of AGE supplementation and at the end of the washout period. *Conclusion:* AGE supplementation caused an increase in the serum levels of the IL-6, IL-10 and HSP27 response and may have an important biological function in protection against oxidative stress and apoptosis by upregulating the levels of these parameters.

Key words: phenolics, aged garlic extract, oxidative stress, antioxidants

Introduction

Aged garlic extract (AGE) is made from organically grown garlic cloves (*Allium sativum L.*) that are sliced and soaked in an aqueous ethanol solution, extracted and aged at least 20 months. The extract is then filtered and concentrated under reduced pressure at low temperature. AGE consists of mainly more water-soluble compounds as opposed to oil soluble compounds. These compounds are reported to have the potential to affect immunity, such as the lectin family, which is known to interact with pathogen recognition receptors on immune cell surfaces (1,2). It has been further reported that AGE reduces cholesterol, oxidative stress, blood pressure, and improves endothelial function (3-5).

IL-6 is an important cytokine featuring redundancy and pleiotropic activity and exerts a central role in host defence against environmental stress, including infection and injury (6). It has been well documented that IL-6 acts as a major pro-inflammatory mediator for the induction of the acute phase response, causing a wide range of local and systemic changes such as fever, leucocytes recruitment and activation, hepatic regeneration and hemodynamic effects (7). Data obtained from clinical and experimental studies indicates that the key role of IL-6 is in mediating the acute phase response, and is a prognostic biomarker in sepsis

and various acute organ injuries. It has been noted that IL-10 is known to be a pleiotropic and potent anti-inflammatory and immunosuppressive cytokine and its expression can be controlled at the transcriptional and post-transcriptional stage. IL-10 secretion can be promoted through direct (e.g., inflammation of the CNS through trauma, neurosurgery, or increased intra-brain pressure) and indirect (e.g., bacteremia) activation of the stress axis promotes. In contrast, oxidative stress in kidneys is associated with decreased IL-10 concentration. It has been reported that IL-10 has an important role in the prevention of infection-related tissue damage (8). It has also been shown to prevent against tissue damage in diabetic wounds, ischemic stroke, and myocardial remodelling (9,10). HSP27 is ubiquitously expressed and has an important role in the regulation of main cellular physiologic functions such as inhibition of apoptosis, protection against oxidative stress, binding erroneously folded proteins for transfer to ATP-dependent chaperones and to the proteasome for further degradation and the regulation of the cytoskeleton (11).

Numerous studies have already shown the protective effect of AGE on chronic disease such as cardiovascular and cardiovascular related disease (12-15). However, the effects of AGE on pro-inflammatory cytokine (IL -6, IL-10) and HSP27 response have not been fully documented in humans. Therefore, the aim of the study to investigate effect of the short term AGE supplementation on the serum cytokines (IL-6 and IL-10) and biochemical parameters (HSP27, TAS, TOS, cortisol and testosterone) in healthy men.

Methods

Participants and procedure

Six sedentary healthy male participants (Mean age 30.2 ± 2.9 year, height 175.6 ± 2.8 cm) participated in this study. The study complied with the principles of the Declaration of Helsinki and was approved by the School of Medicine Balikesir University Ethics Committee and informed written consent was obtained from each volunteer (Ethic Protocol Number 2014/61). The participants who were taking any medicine, antioxidants or other dietary supplementation had any medical history of disease, had a history of allergy or any injuries were excluded from the study. Anthropometric parameters namely BMI (Body Mass Index), percentage of fat percent, fat mass and fat free mass measured by using the Tanita BC 418 MA (Tanita Corporation, Japan).

Biochemical Analysis

Plasma levels of IL-6, IL10 and HSP27 (eBioscience, Austria) were determined by enzyme-linked immunosorbent assay (ELISA) on a diagnostic instrument (BioTek, ELx 800, U.S.A). The amounts of testosterone and cortisol hormone were also analysed on diagnostic instrument. Total antioxidant (TAS) and oxidant (TOS) activities were analysed by spectrophotometry.

AGE supplementation

The supplementation with AGE was performed as described previously by Dillon et al (16). After the baseline test, the participants consumed 5 mL of AGE (taken in a small volume of fruit juice) daily for 10 days between 07.00 and 09.00 a.m.; otherwise, the volunteers followed their usual diet and lifestyle, excluding alcohol intake. This dose of AGE is recommended by the manufacturers and has been used in previous studies, which showed inhibition of ADP-induced platelet aggregation (17).

Results

The results of the study are summarised in Table 1 and Table 2. AGE increased serum levels of IL-6, IL-10 and HSP27 response in the post-supplementation period ($p=0.05$, $p=0.04$, $p=0.001$, respectively, Table 2.). In contrast, as expected the amounts of these cytokines declined in the washout period. However, the serum levels of other biochemical parameters such as cortisol, testosterone, TAS and TOS were not affected by AGE supplementation ($p>0.05$). Interestingly, AGE supplementation for ten days significantly

Table 1. Effect of AGE supplementation on physical characteristics of the subjects.

	Pre Supp Test 1	Post Supp Test 2	Washout Test 3	F	P	Significant difference
Weight (kg)	73.86 (5.52)	73.76* (5.68)	75.10* (5.85)	8.48	0.011	Test 2-3
BMI (kg/m^2)	24 (1.92)	23.98* (1.94)	24.52* (1.95)	8.08	0.012	Test 2-3
Fat Percentage (%)	11.18* (3.95)	12.10 (3.70)	14.02* (3.17)	7.80	0.013	Test 1-3
Fat Mass (kg)	9.02** (3.71)	9.70 (3.58)	11.22** (3.41)	13.80	0.003	Test 1-3
Fat Free Mass	64.8 (5.51)	64.1 (5.69)	63.8 (6.12)	1.44	0.290	

The results are expressed as mean ± standard deviation (SD), (n=6). The significance of differences among all steps of the groups was analyzed by analysis of variance (ANOVA). Significant differences were analyzed between post-supplementation and washout period in experimental sessions. *p<0.05, **p<0.001. Pre-Supp: Pre-supplementation, Post-Supp: Post supplementation.

Table 2. Effect of AGE supplementation on biochemical parameters.

	Pre Supp Test 1	Post Supp Test 2	Washout Test 3	F	P	Significant difference
IL-10 (pg/ml)	3.1 (0)*	60.2 (37.6)	44.1* (16.6)	2.49	0.04	Test 1-2
IL-6 (pg/ml)	5.66 (0.15)	6.14* (0.35)	5.51* (0.64)	11.11	0.05	Test 2-3
HSP27 (pg/ml)	1549.7 (472)	5263 (800)	724.3** (122)	11.11	0.001	Test 1-2, 2-3
Testosterone (pg/ml)	751 (115.7)	614 (115.2)	616.4 (88)	0.55	0.58	
Cortisol (pg/ml)	10426 (174)	10127 (455)	9560 (784)	0.77	0.47	
Total antioxidant status (TAS) (mmol/L)	1.7 (0.76)	1.82 (0.08)	1.75 (0.11)	0.43	0.65	
Total oxidant status (TOS) (mmol/L)	6.36 (0.41)	7.27 (0.43)	6.51 (0.38)	1.26	0.31	

The results are expressed as mean ± standard deviation (SD), (n=6). The significance of differences among all steps of the groups was analyzed by analysis of variance (ANOVA). Significant differences were analyzed between post-supplementation and washout period in experimental sessions. *p<0.05, **p<0.001. Pre-Supp: Pre-supplementation, Post-Supp: Post supplementation.

elevated anthropometric parameters such as body weight (p=0.011), BMI (p=0.012), percentage of fat (p=0.013), fat mass (p=0.003) in the washout period when compared to the post supplementation period; however, free fat mass levels were unchanged in response to AGE supplementation (Table 1).

Discussion

In this experiment, the serum cytokines (IL-6 and IL-10) and biochemical parameters (HSP27, TAS, TOS, cortisol and testosterone) were used to evaluate the effect of AGE supplementation in six sedentary

healthy males. Previous publications have shown that AGE exerted a protective effect against oxidant damage and displayed anti-inflammatory properties (18). It has been reported that administration of AGE causes the inhibition of platelet aggregation in both healthy subjects and subjects with cardiovascular disease (16). In our study AGE supplementation had no effect on serum TAS and TOS level. A few studies showed that administration of garlic supplements increased in serum TAS level after 4 weeks in healthy volunteers (19). Moreover, Avci et al. demonstrated that garlic supplementation decresed level of the erythrocyte MDA levels and also increasing antioxidant enzyme activitiy in elderly population (20). Additionally, Koseoglu et al. (2010) reported that a significant increase in serum TAS was detected at 15 days (%7) 30 days (%9) compared with 3 h and control in healthy subjects. These differences are uncertain that a 7% and 9% increase in serum TAS might have a beneficial physiological impact (21). In our study we had a supplemented the subjects in 10 days, this supplementation period less than the 15 days. Importantly, the appearance of serum TAS increasing the effect of AGE supplementation, at least 15 days. It should be suggested that the supplementation period is important.

Our results indicate that 10 days of dietary AGE supplementation significantly increased plasma levels of IL-6and IL-10 and HSP27 response. As far as we are aware this is the first study investigating the effects of AGE supplementation on IL levels, and HSP response in healthy human subjects. There are a few studies indicating the anti-fatigue effects of garlic supplementation *in vivo* models and these have shown that AGE significantly improves running times in rats (22). Another study reported that six week of AGE oil supplementation reduced the heart rate at peak exercise and the work load of the heart in cardiac patients (23).

In the present study AGE increased IL-6, IL-10 and HSP27 response in the post-supplementation period. This observation has not been reported before and IL-6 and IL-10 are cytokine and they act on a variety of tissues, and has both pro-and anti-inflammatory properties (24). The plasma level of IL-6 and IL-10 are within the physiological range, although these levels were increased in the post-supplementation period. It has been well documented that the exercise induces plasma IL-6 levels which has inflammatory properties and also plays an anti-inflammatory role within the body (25,26). In addition, IL-10 has an important role in the prevention of infection-related tissue damage (8). Increased IL-10 concentrations have been shown to protect against damage to tissues such as diabetic wounds, ischemic stroke, and myocardial remodelling (9,10). Limited study showed that garlic supplementation was increased IL-8 and IL-12 levels compared to baseline levels in healthy subjects as an acute response (27). In addition, AGE supplementation increases in the blood flow in healthy subjects, it may be associated significant elevation of plasma level of IL-6 (28). Moreover, the improvement in blood flow was significantly related to, and therefore possibly mediated by, increases in the circulating levels of IL-6. Interleukin-6 and IL-10 is a multifactorial cytokine that plays an important role in host defences and immune responses. Furthermore, HSP27 is also ubiquitously expressed and takes place in the regulation of main cellular physiologic functions such as inhibition of apoptosis, protection against oxidative stress, binding erroneously folded proteins for transfer to ATP-dependent chaperones and to the proteasome for further degradation and the regulation of the cytoskeleton (11).

Interestingly, AGE supplementation for ten days significantly elevated body weight in subjects. The subjects subjectively reported increased appetite throughout the study period, therefore, they may be put on weight. On the contrary, one study evaluated the effect of garlic powder supplementation for 3 months improve insulin resistance, fatty liver index, metabolic syndrome and reduce appetite (29). There is no clear evidence the effect on garlic on appetite. The mechanism in this field is unclear.

Conclusion

AGE supplementation upregulated levels of IL-6 and IL-10 and HSP27 response and may have a protective role against the oxidative stress and apoptosis. There were some limitations in this pilot study as it only involved six participants hence a larger cohort is required to validate the data.

Declaration of Conflicting Interests: The authors declare that they have no conflict of interest.

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