
Anti-allergic effects of aged garlic extract

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Summary

To examine the effect of Aged Garlic Extract (AGE) on the function of mast cells and activated T lymphocytes, we adopted the *in vitro* histamine release system, the *in vivo* IgE mediated skin reaction system and the *in vivo* late phase reaction system. Consequently, at 1.25, 2.5, and 5.0% (v/v), AGE dose-dependantly inhibited the antigen specific histamine release by mouse anti-TNP monoclonal antibody and TNP-BSA hapten carrier complex against rat basophil cell line RBL-2H3 by 50, 80, and 90 percent, respectively. In the IgE mediated skin reaction system, repeated or single intragastric administration of AGE (10 ml/kg), decreased by 25–45% the antigen specific ear swelling which was induced by a picryl chloride ointment applied to the ear of mice also given an intravenous administration of anti-TNP antibody IgE ascites. In the late phase reaction system, repeated or single intragastric administration of AGE (10 ml/kg) suppressed by 45–55% the antigen specific ear swelling induced by a secondary challenge to the ear of mice given a picryl chloride ointment seven days prior.

These results suggest that AGE application could modify, directly or indirectly, the function of mast cells, basophils and activated T lymphocytes which play a leading role in allergic cascade reactions including inflammation.

Key words: Aged Garlic Extract (AGE), rat basophilic cell line (RBL-2H3), histamine release, IgE, skin reaction, late phase, atopy, atopic dermatitis, allergy.

Introduction

In atopic dermatitis, the basic mechanism underlying skin lesion formation has remained largely speculative, except for the delayed hypersensitivity reaction against environmental substances (Mitchell, 1982, Uehara, 1976, Adinoff, 1988) or the IgE mediated immediate hypersensitivity reaction against food or inhalant or mite derived allergens (Atherton, 1978, Sampson, 1988, Kolmer, 1995). The pathogenesis of atopic dermatitis may be more easily explained by a model of delayed skin reactions which occur after mast cell activation. Whereas in pollen-induced allergies, skin lesion formation appears to be caused by the IgE mediated immediate

hypersensitivity reaction triggered by mast cells (Van Bever, 1993). Each of these allergic cascade reactions involves the activity of mast cells, basophils, neutrophils, eosinophils, T lymphocytes and macrophages (Leung, 1995).

Garlic is a natural food which has shown some historical, though anecdotal effects in the treatment of allergies. An Aged Garlic Extract (AGE) has demonstrated an array of pharmacological effects, including immunomodulatory effects, in studies (Jain, 1978, Lau, 1986, Abdullah, 1989). Beyond one report of the inhibitory effects of garlic against β -hexosaminidase release from rat basophilic leukemia cells (RBL-2H3) (Tanaka, 1992), little is known scientifically about anti-allergy effects of garlic. Therefore, in this study, to examine the effect of AGE on the function of mast cells and activated T lymphocytes, we adopted the *in vitro* histamine release system (Teshima, 1994), the *in vivo* IgE

Abbreviations: FBS – fetal bovine serum; BSA – bovine serum albumin; CMC Na – sodium carboxymethylcellulose; TNP – 2,4,6-trinitrophenol; TNBS – 2,4,6-trinitrobenzenesulfonic acid; PBS – phosphate buffered saline

mediated eczematous skin reaction system (Katayama, 1990) and the *in vivo* late phase reaction system (Solley, 1976).

Material and Methods

Animals. BALB/c mice (male, 7 weeks old) and Wistar rats (male, 7.5 weeks old) were purchased from Charles River Japan Inc. (Yokohama, Japan).

Cells. Mouse anti-TNP specific IgE monoclonal antibody producing hybridoma IGEL-2a and rat basophil cell line RBL-2H3 (Tanaka, 1992) were purchased from American Type Culture Collection. These cell lines were maintained in RPMI 1640 containing 10% FBS.

Aged Garlic Extract (AGE). AGE was prepared by Wakunaga Pharmaceutical Co., Ltd. AGE manufactured under license issued by the Ministry of Health and Welfare of Japan was formulated using the following steps: sliced raw garlic (*Allium sativum* fa. *pekinense*) was dipped into aqueous ethanol and extracted over 10 months at room temperature (Imai, 1991). Extracted solids in AGE were approximately 15 percent (150 mg/ml). AGE used for these experiments contained S-allylcysteine in the range of 1.6–2.4 mg/g (calculated as dry weight) (Moriguchi, 1996).

Chemicals. TNBS, picryl chloride (2,4,6-trinitrochlorobenzene), were purchased from Tokyo Kasei Kogyo Co., Ltd. (Tokyo, Japan). Oxatomide (Cellect Tablet) was purchased from Kyowa Hakkou Kogyo Co., Ltd. (Tokyo, Japan). Dexamethasone purchased from Sankyo Co., Ltd. (Tokyo, Japan). Histamine, perchloric acid, *o*-phthalaldehyde and CMC Na were purchased from Wako Pure Chemical Co., Ltd. (Tokyo, Japan).

Histamine release system (Teshima, 1994). For the target cell, rat basophil cell line RBL-2H3 (Tanaka, 1992) was inoculated to 24 well plates (Falcon) at 2×10^5 cells/ml. After 24 hours, these cells were washed twice with PBS(–). Since the homology of IgE protein between mice and rats is more than ninety-five percent, mouse IgE can bind to rat IgE receptors. So, for the first reaction, 0.3 ml of the culture supernatant (IGEL-2a mouse hybridoma including anti-TNP mouse monoclonal IgE antibody) was added to these cells to occupy the IgE receptors of RBL-2H3. After three hours, cells were again washed twice with PBS(–). For the second reaction, 0.3 ml of 10 mg/ml TNP-BSA (Garvey, 1977) in Tyrode solution was added as the hapten-carrier antigen to induce the release of granules from RBL-2H3. AGE or oxatomide were applied after the 1st reaction and before the 2nd reaction. After one hour, released histamine in the culture supernatant from RBL-2H3 was measured by the *o*-phthalaldehyde method (Teshima, 1994). In this system, 25% of the histamine in RBL-2H3 was released by the antigen-antibody specific interaction, that was 116 ± 7 ng/ml, and spontaneous released histamine was 36 ± 4 ng/ml.

IgE mediated skin reaction system (Katayama, 1990).

Anti-TNP IgE antibody ascites was prepared by intraperitoneal administration of IGEL-2a mouse hybridoma (Tanaka, 1992) to BALB/c male mice and the titer of PCA was examined. So, for the first treatment, BALB/c male mice were given an intravenous administration of anti-TNP IgE antibody ascites (PCA titer was 1:4800, 0.5 ml/mouse). After one hour, for the second treatment, 0.3% picryl chloride solution (15 μ l/ear) in 4:1 acetone olive oil were applied on both sides of the right pinna of the mice. Picryl chloride, following binding to the amino group of skin tissue or protein, can function as the hapten-carrier which bridges IgE antibodies occupying the IgE receptors (Katayama, 1985). These treatments induced spongiotic changes in the skin on the ears of the mice. The left pinna was untreated as a control. Ear thickness was measured by a dial thickness gauge (minimum scale was 0.01 mm, range of measurement was 0–10 mm). At four hours prior to picryl chloride treatment, the ear thickness of the mice was measured. At three hours prior to picryl chloride treatment, IgE was applied to the mice. And at 1, 4 and 24 hours after picryl chloride treatment, ear thickness of the mice was again measured three times. AGE or oxatomide were applied at single (one hour prior of picryl chloride treatment) or repeated (21 and 1 hours prior, 3 and 20 hours later of picryl chloride treatment) increments by oral administration.

Late phase reaction system (Solley, 1976). For the first treatment, 7% picryl chloride solution in ethanol (100 μ l/mouse) was rubbed on the abdominal skin of BALB/c male mice. After seven days, for the second treatment, 1% picryl chloride solution in olive oil (10 μ l/ear) was applied to both sides of the right pinna of the mice. These treatments induced spongiotic changes in the skin on the ears of the mice. The left pinna was untreated as a control. At one hour prior and at 24 hours later of the second treatment, ear thickness of the mice was measured twice. AGE or dexamethasone was administered intragastrically three times at 0, 4 and 16 hours after the second treatment.

Statistics. Statistics were calculated using the Student's *t*-test or the Aspin-Welch method after improvement of F-test, where applicable.

Results

Inhibition of histamine release by AGE application

In the first experiment, we examined whether AGE could inhibit the antigen specific histamine release induced by mouse anti-TNP monoclonal antibody and TNP-BSA hapten carrier complex against the rat basophil cell line RBL-2H3. In this system, 25 percent of the histamine in RBL-2H3 was released by antigen (TNP-BSA)-antibody specific interaction (116 ± 17 ng/ml), and spontaneously released histamine was found to be 36 ± 4 ng/ml. Oxatomide (10 μ g/ml of RPMI 1640), an anti-allergy drug, inhibited histamine release by 80 percent, whereas AGE inhibited hista-

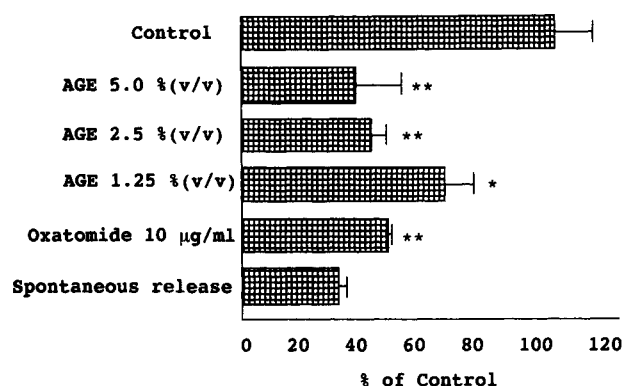


Fig. 1. Histamine release inhibition by AGE application. In this system, 25% of the histamine in RBL-2H3 was released by antigen-antibody specific interaction (control), that was 116 ± 17 ng/ml, and spontaneous release was 36 ± 4 ng/ml. Values are given for percent of control as mean $\pm$ SD ($n = 6$). Asterisk denotes significant difference from the control, *; $p < 0.05$, **; $p < 0.01$.

mine release in a dose dependent manner at 1.25, 2.5, and 5.0% (v/v), respectively by 50, 80, and 90 percent (Fig. 1).

Decreased IgE mediated skin reaction inducing antigen specific ear swelling by AGE application

In the second experiment, using an *in vivo* type I allergic reaction experimental model, we examined whether AGE could decrease the antigen specific ear swelling induced by application of picryl chloride ointment to the ear of mice which were given an intravenous administration of anti-TNP IgE antibody ascites.

Results of repeated oral administration of oxatamide and AGE in the *in vivo* type I allergic reaction experimental model can be found in Fig. 2. For the control group (0.5% CMC Na, 10 ml/kg), ear swelling was observed on the right pinna to which picryl chloride was applied. The ear thickness was 9.32×10^{-2} mm one hour after the antigen challenge, 7.21×10^{-2} mm after four hours, and 7.04×10^{-2} mm after 24 hours. Ear swelling appeared to occur due to degranulation of mast cells one hour after the antigen challenge. The late phase of immune reaction, in which eosinophils and lymphocytes participate after being induced by chemical mediators of mast cells, was not observed in the control group until after 48 hours (data not shown). In the oxatamide group (30 mg/10 ml of 0.5% CMC Na/kg), the ear swelling was decreased by 58 percent in the control group ear one hour after the antigen challenge and by 57 percent four hours after the antigen challenge. The AGE group (10 ml/kg) was found to be slightly inferior to oxatamide. Ear swelling was decreased by 25 percent in the control group ear one hour after the antigen challenge and by 45 percent four hours after the antigen challenge. Therefore, AGE was shown to have an ability to decrease *in vivo* allergic reactions triggered by mast cells.

The results of single intragastric administration of oxato-

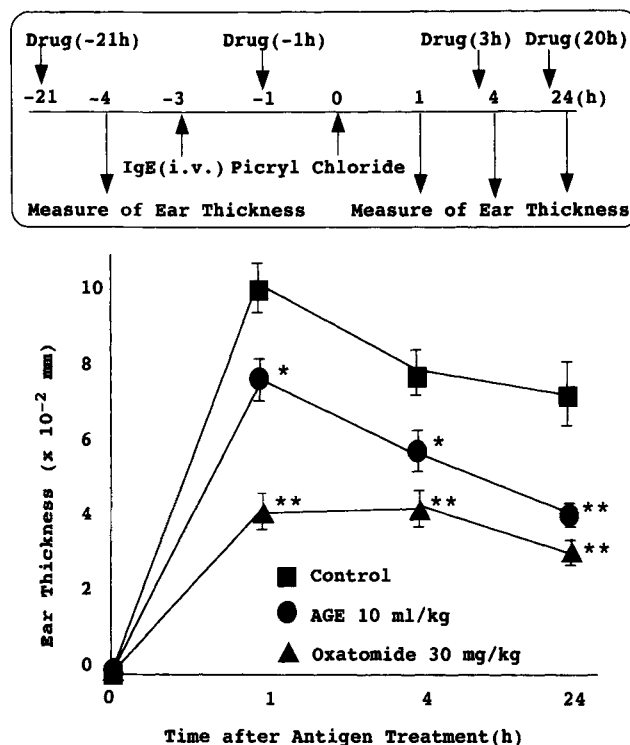


Fig. 2. Decreased IgE mediated skin reaction inducing antigen specific ear swelling by repeated application of AGE. AGE or oxatamide was applied repeated (21 and 1 hours prior, 3 and 20 hours later of picryl chloride treatment) increments by oral administration. Ear thickness was measured by a dial thickness gauge. Values are given as mean $\pm$ SE ($n = 10$). Asterisk denotes significant difference from the control, *; $p < 0.05$, **; $p < 0.01$.

mid and AGE can be found in Fig. 3. One hour following application of picryl chloride, ear swelling was decreased by 47 percent in the oxatamide group and by only 19 percent in the AGE group. On the other hand, four and 24 hours following application, AGE out-performed oxatamide.

Suppressed late phase reaction inducing antigen specific ear swelling by AGE application

In the third experiment, using an *in vivo* type IV allergic reaction experimental model, we examined whether AGE could suppress the antigen specific ear swelling induced by a picryl chloride secondary challenge to the ear of mice which were given a picryl chloride ointment to the abdominal skin seven days before. Either dexamethasone, a so-called immunosuppressor, or AGE were administered orally 0, 4, and 16 hours after the secondary challenge (three times or one time at each respective hour). Ear thickness was then measured 24 hours after the secondary challenge. As shown in Fig. 4, ear thickness in the control group (water, 10 ml/kg) was 6.53×10^{-2} mm 24 hours after the secondary challenge. In the dexamethasone group (0.1 mg/10 ml of water/kg), ear swelling was suppressed by 65 percent, whereas in the AGE (10 ml/kg) group, ear swelling was

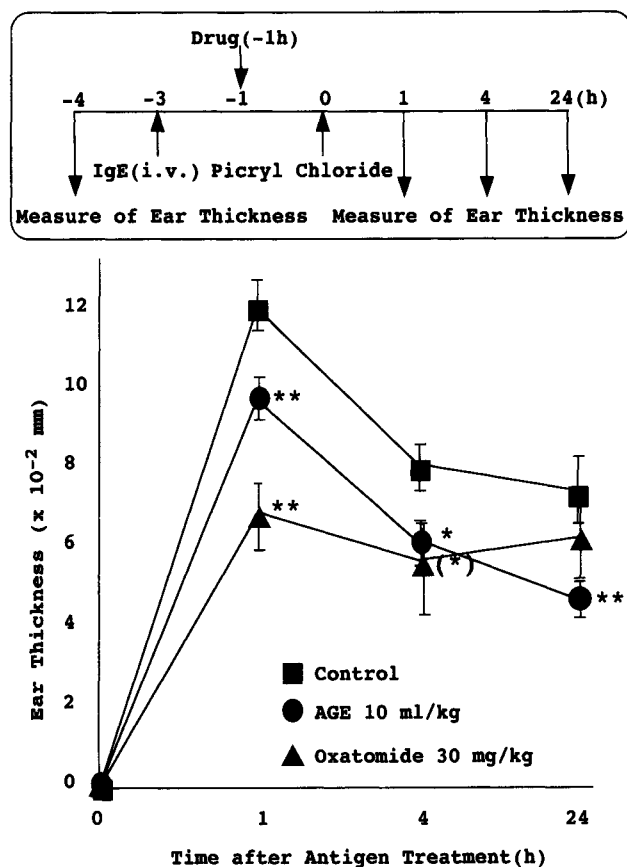


Fig. 3. Decreased IgE mediated skin reaction inducing antigen specific ear swelling by single application of AGE. AGE or oxatomide was applied one hour prior of picryl chloride treatment by oral administration. Ear thickness was measured by a dial thickness gauge. Values are given as mean $\pm$ SE ($n = 10$). Asterisk denotes significant difference from the control, (*); $p < 0.1$, *; $p < 0.05$, **; $p < 0.01$.

suppressed by 55 percent. Moreover, following the single administration of AGE, four hours after the secondary challenge, swelling decreased by 40 percent. The other single application had no effect.

Discussion

In our research, we have demonstrated anti-allergic effects of AGE by adopting *in vitro* and *in vivo* allergic experimental models. First, in the histamine release system, AGE inhibited the antigen specific histamine release by mouse anti-TNP monoclonal antibody and TNP-BSA hapten carrier complex against the rat basophil cell line RBL-2H3. AGE exerted almost the same inhibitory effect as oxatomide. Oxatomide is a benzimidazolone compound. It appears to function by suppressing extra-cellular calcium ion influx and intra-cellular calcium ion mobilization. This results in the suppression of chemical mediator release, such as histamine, leukotriene, and prostaglandin D₂ (Awouters, 1977). Therefore, our results sug-

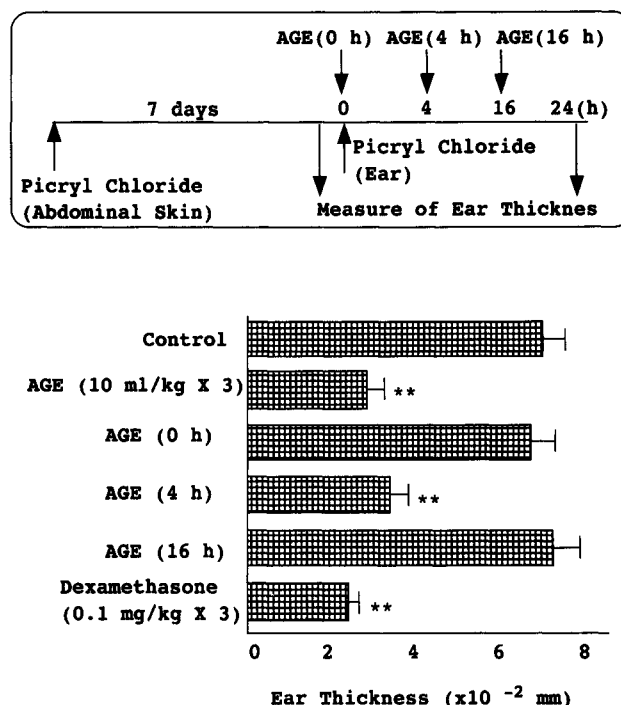


Fig. 4. Suppressed late phase reaction inducing antigen specific ear swelling by AGE application. AGE or dexamethasone was applied 0, 4, and 16 hours later the secondary challenge (three times or one time at each respective hour) by oral administration. Ear thickness was measured by a dial thickness gauge. Values are given as mean $\pm$ SE ($n = 10$). Asterisk denotes significant difference from the control, **; $p < 0.01$.

gest that AGE contains constituents that function similar to oxatomide.

Second, in the IgE mediated skin reaction system, AGE decreased the antigen specific ear swelling induced by application of a picryl chloride ointment to the ear of mice which were given an intravenous administration of anti-TNP antibody IgE ascites. The decreased percentage of ear swelling following single administration corresponded to that found following repeated administration. Further, AGE application, especially one hour after picryl chloride application, was most effective at decreasing ear swelling. Taken together, it appears critical that AGE constituents be present in the body to be effective at inhibiting an allergic response when allergens bind to allergen specific IgE antibody on the surface of IgE receptor bearing cells.

Third, in the late phase reaction system, AGE suppressed the antigen specific ear swelling induced by a secondary challenge to the ear of mice which were given a picryl chloride ointment seven days before. The second single administration of AGE four hours after secondary challenge was found to be the most effective. In the late phase reaction system, activated T lymphocytes release various cytokines several hours after the secondary challenge, and subsequently these cytokines induce the accumulation of several inflammatory cells to the antigen location. Additionally, in

the pathogenesis of atopic dermatitis, IgE plays an important role, and IL-4 producing Th2 cells have been ascribed a key role in allergic diseases such as atopic dermatitis. Therefore, it appears that AGE modulates production or release of cytokines from activated T lymphocytes.

Recently, several important advances in our understanding on the immunopathogenesis of atopic dermatitis have been highlighted (Leung, 1995). These include IgE-mediated degranulation of mast cells or basophils, activation of monocytes, and stimulation of Th2 cells. IL-4 producing T lymphocytes play a predominant role in the acute onset of skin inflammation. On the other hand, macrophages and eosinophils play a dominant role in the chronic onset of skin inflammation. Effector cells, which may be involved in the chronicity of atopic dermatitis, produce inflammatory cytokines such as IL-2 (Agata, 1992; Itoh, 1985), IL-4 (Thepen, 1996; Lester, 1995), IL-5 (Ishii, 1996), IL-6 (Lester, 1995), IL-8 (Kimata, 1994), IL-10 (Ohmen, 1995), GM-CSF (Leung, 1995), interferon-gamma (Jujo, 1992) and prostaglandin E2 (Chan, 1996). Additionally, keratinocyte damage, induced by *Staphylococcus aureus*, is accompanied by the release of inflammatory cytokines (Strange, 1996). As AGE modulated the functions of mast cells in the early phase and T lymphocytes in the late phase, it may contribute to mitigate the condition of a patient at some critical point in the pathogenesis of atopic dermatitis.

New immunologic therapies for chronic allergic disease in the future are likely to involve the induction of modulation of Th2 cells and their cytokines targeting specific organs. As our understanding of the immunopathogenesis of allergic responses continues to grow, manipulation of the immune response in atopic dermatitis and other allergic disease is likely to take an exciting new direction in the treatment of this common group of illness (Leung, 1995). Whereas, garlic is a common plant used mainly as a food item, in many different cultures of the world it is deemed as a medicinal herb (Lau, 1983; Adetumbi, 1983). As recorded around 1550 B.C. in the Ebers Papyrus (Block, 1985), garlic was used externally for the treatment of tumors by ancient Egyptians and internally by Hippocrates and Indian physicians (Hartwell, 1971). Lau (1991) and Morioka (1993) reported the possibility that AGE or its constituents might modulate host immune response. Since AGE contains constituents which relieve the symptoms in both type I and type IV allergy experimental models, we propose the possibility that constituents in AGE may exert anti-allergic effects. The data from the present study provide additional evidence to support the hypothesis that AGE constituents may indeed modulate the host immune network.

In conclusion, it was clearly demonstrated that AGE application could modify, directly or indirectly, the function of mast cells, basophils and activated T lymphocytes which play a leading role in allergic cascade reactions including inflammation. In medical therapy, steroids are mainly used

for atopic dermatitis. However, they have several side effects. More desirable, of course, would be drugs which have no side effects. AGE has been confirmed to be very safe by several toxicity studies, both clinical and in animals (Nakagawa, 1980; Sumiyoshi, 1984; Yoshida, 1984), and these encouraging data may suggest that allergy diseases can be moderated by daily intake of AGE.

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