THE PROTECTIVE EFFECTS OF AQUEOUS GARLIC EXTRACT AGAINST WATER AVOIDANCE STRESS-INDUCED MAST CELL DEGRANULATION IN THE DERMIS

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ABSTRACT

Objective: The aim of the present study was to investigate the effects of aqueous garlic extract (AGE) on water avoidance stress (WAS)-induced degranulation of mast cells in the dermis.

Material and Methods: Wistar albino rats were exposed to WAS (WAS group). After exposing the animals to WAS (WAS plus AGE group), 250mg/kg AGE was injected i.p.. Dermal mast cells were stained with toluidine blue and investigated using light microscopy.

Results: The number of both granulated and degranulated mast cells was higher in the WAS group, when compared to the control group. The number of mature granulated and degranulated mast cells was lower in the WAS plus AGE group when compared to the WAS group.

Conclusion: Chronic AGE treatment reduced WAS-induced infiltration and activation of mast cells in the dermis and may provide a useful therapeutic option in stress-induced skin disorders.

Keywords: Dermal, Mast cell, Water avoidance stress, Aqueous garlic extract

INTRODUCTION

Stress-related disturbances of homeostasis and induction of the pathogenesis of various diseases are well established. Physical and emotional stress can be etiological factors underlying several diseases. Stress can induce inflammatory responses in various organs and when stress is chronic it may...
induce or aggravate chronic inflammatory diseases such as migraine, atopic dermatitis, urticaria and psoriasis. The role of mast cells in inflammatory and allergic reactions including stress-induced inflammatory diseases is well documented. Mast cells are implicated in skin disorders such as atopic dermatitis, psoriasis and these diseases are either induced or worsened by stress. The skin of patients with atopic dermatitis contains significantly higher numbers of mast cells than intact skin. In psoriasis, the number of mast cells is significantly elevated in both lesional skin and intact skin. Mast cells release inflammatory mediators upon activation, such as histamine, proteases, prostaglandins, leukotrienes and cytokines that have potent vasodilatory, and inflammatory properties. For example, histamine from mast cells increases vascular permeability upon activation and stimulates cutaneous sensory nerves. Stimulation of unmyelinated sensory nerves leads to the release of neuropeptides such as substance P which act on mast cells, blood vessels and leukocyte infiltration. It has been recently suggested that the skin may have its own equivalent of the hypothalamic–pituitary–adrenal (HPA) axis because the corticotropine releasing hormone (CRH) and its receptors were shown to be present in the skin.

Many beneficial, health-related biological properties are attributed to garlic, among them, that garlic preparations including aged garlic, prevent tumour promotion, cardiovascular diseases, liver damage and aging which are considered to be associated with oxygen radical and lipid peroxidation. The intrinsic antioxidant activity of garlic, garlic extracts and some garlic constituents have been widely documented in vivo and in vitro.

Considering the fact that psychological stress induces or worsens various skin conditions, we investigated whether water avoidance stress (WAS) affects the occurrence of mast cells in the skin and their degranulation and if so, whether aqueous garlic extract can reduce mast cell activation.

**METHODS**

**Animals:** Adult female Wistar albino rats weighing 200-250 g were housed individually in a light- and temperature-controlled room on a 12/12 h light/dark cycle, and fed a standard pellet lab chow.

**Stress Procedure:** The rats were handled daily by the same investigator for 2 weeks before the start of the study and were then submitted to WAS as described previously. The procedure was as follows: rats were placed on a glass platform (8X6 cm) in the middle of a plastic container with a diameter of 90 cm and height of 50 cm filled with water of 25ºC to 1 cm below the level of the platform. The rats avoided the aversive stimulus (water) by staying on the platform for 2 h. All experimental procedures were performed between 8:00 and 10:00 to minimize the effect of circadian rhythms.

**Preparation of aqueous garlic extract:** Peeled garlic (30 g) was crushed with distilled water in a mortar. The crushed material was carefully decanted by pressing and 60 ml of aqueous extract was extracted. One milliliter of aqueous extract contained 500 mg of garlic materials.

**Experimental groups:** Three groups of rats were treated as follows: 1) Control group (n=8): rats were placed on the platform in a container without water for 2 h and were injected with a saline solution 2) Stress (WAS) group (n=8): rats were exposed to WAS for 2 h daily for 5 days after receiving an injection of a saline solution each day. 3) Stress exposed, AGE treated group (WAS+AGE group) (n=8): After exposing them to WAS, 250mg/kg AGE was injected ip. for 3 days into the animals. During the experimental procedures the animals were fed with a standard pellet lab chow and water ad libitum. At the end of the experiments, the rats were euthanized by decapitation.

**Histological preparations:** Pieces of skin from the back of approximately 2 cm² (skin were taken from the same location in all rats) including the hypodermis and were fixed in 10% formaldehyde and then routinely processed for paraffin embedding. Paraffin
sections (5-µm thick) were stained with 1% toluidine blue (Merck, 1190322). The sections were examined at 400X magnification with a BX50 photomicroscope (Olympus, Tokyo, Japan).

**Mast cell counting and statistical analysis:** Sections of the skin were evaluated for mature granulated mast cells and degranulated mast cells. Granulation was considered according to the fullness of the mast cells with granules whereas degranulation meant when the granules were out of the cell and observed nearby the cells. Ten randomly taken sections (the first, eleventh, twenty-first, etc) from each animal were selected and the mast cells were counted without knowing to which treatment group the rat belonged. In each section, mast cells containing metachromatic granules were counted separately at 400x magnification in five consecutive dermal areas by one or more observer and the observers were blinded to the treatment of the animals. An eye-piece graticule (size 0.0785 mm²) was used in order to avoid overlap of areas to be counted. It had a vertical and a horizontal axis, so that the area was divided into 4 parts, which facilitated counting. Areas selected in each region were surveyed for mast cells and the mast cell density was then expressed as cell number per unit area. The data were analyzed by using one-way analysis of variance (ANOVA). Differences between groups were determined with the Dunnett’s multiple comparisons test and the data were expressed as mean ± standard error of the mean. The significance of differences was taken as the level p < 0.05.

**RESULTS**
In the control (Fig.1a) mast cells stained with toluidine blue were mostly intact. In the WAS group (Fig 1b) both the granulated and degranulated mast cells were increased in number. In the WAS + AGE group (Fig. 1c) most mast cells were granulated with a darkly stained cytoplasm and a few of them were degranulated. Quantitative analysis of the numbers of mast cells that were granulated and degranulated in the different treatment groups is shown in Fig. 2. According to the statistical results the numbers of mast cells were increased in the WAS group, and numbers of activated mast cells were increased in the WAS group when compared with the control groups. Numbers of both granulated and degranulated mast cells were significantly reduced in the WAS + AGE group when compared with the WAS group (Fig. 2).

**Fig. 1:** Photomicrographs of rat dermis of the toluidine blue staining showing granulated and degranulated mast cells. Only granulated mast cells (¢) were present in the dermis of control (a) rats. Increased numbers of both granulated (¢) and degranulated (inset) mast cells were present in the WAS group (b). Decreased number of both granulated (¢) and degranulated (inset) mast cells were present in WAS + AGE groups (c). Original magnifications: x200, insets: x400
DISCUSSION
The results of the present study demonstrate that AGE has a protective effect on chronic psychological, nontraumatic stress-induced numbers of mature mast cells in dermis of rats. WAS induces psychological stimuli and eWAS may be a model of “life stress” as its duration is five days24.

Psychological and physical stress has been shown to suppress immune functions. Yokoyama et al25 reported that an AGE preparation containing vitamins B-1 and B-12 restored physical stress–induced immune suppression in mice. Recently, it was determined that the effect of AGE on immune suppression caused by psychological stress26,27 is determined.

Activation of mast cells has been shown in different stress models. Repetitive exposure to odors28, Pavlovian conditioning29 and isolation stress conditions30 cause secretion of histamine from mucosal mast cells. We have previously observed that stress-induced inflammatory changes such as mast cell degranulation are mediated by capsaicin-sensitive sensory neurons in the dermis31. Recently, it has been reported that acute immobilization stress also induces mast cell degranulation in the skin, which may be linked to various inflammatory skin diseases32.

A number of reports have revealed the relationship between foods and allergic responses. Tanaka et al33 reported that Allium vegetables, including garlic, inhibit the release of β-hexosaminidase, which is correlated with histamine release, in rat basophilic leukemia cells (RBL-2H3), suggesting an anti-allergic effect34. The anti-allergic activity of (Z)-ajoene might be due to its inhibition of chemical mediator release. It was also found that AGE has an anti-allergic property35. Histamine release in the rat basophil cell line RBL-2H3 was induced by mouse anti-trinitrophenyl (TNP) monoclonal antibody and the TNP-bovine serum albumin (BSA) hapten carrier complex. AGE added to the culture medium at doses of 1.25, 2.5 and 5.0 g/100 g significantly inhibited the antigen specific histamine release by 50, 80 and 90%, respectively. Oral administration of AGE (10 mL/kg) also decreased 25–45% of the ear swelling, used as an index of immunoglobulin (Ig) E–mediated skin reaction, caused by intravenous administration of anti-TNP IgE antibody and subsequent picryl chloride painting on the ear in mice27. In the present study, WAS induced an increase in number and degranulation of mast cells in the rat.
dermis. These results suggest that WAS-induced degranulation of mast cells occurs via activation of inflammatory reaction and, stimulation of mast cell to give anti allergic compounds such as histamine to the extracellular matrix and these can be prevented by treatment with exogenous AGE via modulation of immune, antiinflammatory and antiallergic reactions.

In conclusion, systemic treatment of AGE appears to reduce the WAS-induced increase in numbers and degranulation of mast cells in the skin. Further studies on this subject must be carried out in order to establish the effects of AGE as a possible therapeutic agent in many skin diseases such as psoriasis and atopic dermatitis.

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