Allyl isothiocyanate-induced changes in the distribution of white blood cells in rats

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ABSTRACT — The main pungent component of wasabi (Eutrema japonica) is known to be isothiocyanate and its derivatives, volatile substances. Allyl isothiocyanate (AITC) accounts for more than half of isothiocyanate derivatives. However, there is little information on the effects of AITC on the immune system by analyzing the number of white blood cells (WBCs) over the course of days of AITC administration. In the present study, we studied the effects of AITC (dose=20 mg/kg body weight/day for 10 days, subcutaneous: s.c.) on the number of WBCs (total WBCs, lymphocytes, monocyte, neutrophil, basophil and eosinophil) and plasma corticosterone concentrations in adult male rats. Administration of AITC decreased significantly the number of total WBCs on days 1-4 post s.c. injection by 25-27%. Administration of AITC also decreased the number of lymphocytes on days 1-10 by 21-36% and monocyte on days 1-8 by 28-78%. However, administration of AITC increased the number of neutrophil on days 8-10 by 61-112%. AITC did not change the number of eosinophil and basophil. Plasma corticosterone concentrations during the experimental period were 4.7-8.4 times significantly higher in the AITC group than in the control group, indicating that AITC induced stress-responses. The relative weights of thymus and adrenals per body weight were significantly lower and clearly higher in the AITC group than in the control group, respectively. These results suggest that AITC-mediated stress-responses are at least in part attributable to changes in the number of circulating WBCs.

Key words: Allyl isothiocyanate, Wasabi, White blood cells, Lymphocytes, Plasma corticosterone

INTRODUCTION

Wasabi (Eutrema japonica) is the popular spice grown in Japan and it has been used as condiments and seasoning for dishes such as sushi, sashimi and soba (Kinase et al., 2000). Wasabi has analgesic effect and has been used for reduction of pain caused by rheumatism and neuralgia (Kinase et al., 2000; Tapsell et al., 2006). The main pungent component of wasabi is known to be isothiocyanate (ITC) and its derivatives, volatile substances, and allyl isothiocyanate (AITC) accounts for more than half of ITC derivatives (Iwasaki et al., 2008; Venkatachalam and Montell, 2007). AITC content of wasabi is known to be about 100-150 mg per 100 g (Imaizumi et al., 2010).

It is well known that intake of pungent components of wasabi enhance sympathetic nervous activities and energy metabolism (Iwasaki et al., 2008; Tapsell et al., 2006). In general, transient receptor potential (TRP) cation channels are deeply related to these physiological responses (Venkatachalam and Montell, 2007). Especially transient receptor potential ankyrin 1 (TRPA1) has a central role in the pain response to endogenous inflammatory mediators and volatile irritants, including AITC, cinnamaldehyde (cinnamon) and allicin (garlic), and noxious cold (< 17°C) (Maher et al., 2008). TRPA1 is highly expressed in sensory neurons of the dorsal root, trigeminal and nodular ganglia, and hair cells of the inner ear (Maher et al., 2008). TRPA1 is expressed on the protein level in several non-neuronal human tissues including gastrointestinal mucosa of small intestine and colon. In addition, TRPA1...
is also expressed in duodenal mucosa (Puhonen et al., 2008).

Recently, we reported the effects of capsaicinoids such as dihydrocapsaicin and capsaicin on the number of white blood cells (WBCs) as an index of the immune-responses of body defence system (Akimoto et al., 2009; Aritoshi et al., 2010; Hasegawa et al., 2010; Hwang and Lee, 2006). We also showed that AITC significantly decreased dose-dependently the number of lymphocytes and their subsets (T-lymphocyte and B-lymphocyte) without changing the number of natural killer (NK) cells in rats (Imaizumi et al., 2010). However, the effects of AITC on the immune responsible properties, especially the number of total WBCs, lymphocytes, monocyte, neutrophil, eosinophil and basophil remain relatively unknown. In the present study, therefore, we studied the effects of AITC on the numbers of WBCs, and plasma corticosterone concentrations in rats. In addition, the effects of AITC on the relative weight of stress-responsive thymus, spleen and adrenals per body weight were also examined.

MATERIALS AND METHODS

Experimental protocols and animal care

The experimental protocol is shown in Fig. 1. Male 7-week-old Sprague-Dawley rats (CLEA Japan, Tokyo, Japan) were prefed for 5 days to allow adaptation to new environments (Fig. 1). The rats were housed in cages at a temperature between 23-25°C and relative humidity of 45-60%. Lighting was automatically provided from 8:00-20:00. Animal chow (CE-2, CLEA Japan) and once-boiled tap water were given to the rats ad libitum. After the adaptation period, the rats were randomly divided into the AITC and control groups. The experiments were designed to minimize pain and discomfort for the animals (Imaizumi et al., 2010). All experiments were approved by the Animal Ethics Committee, Waseda University. This study was carried out in accordance with the “Guiding Principles for the Care and Use of Animals in the Field of Physiological Sciences” of the Physiological Society of Japan (2002).

Administration of AITC to rats

AITC (Wako Pure Chem Ind, Osaka, Japan) was dissolved in 2% ethanol, and then added 10% Tween 80 and 0.9% NaCl as a vehicle to obtain 1.0% concentrations of AITC (Imaizumi et al., 2010; Lewerenz et al., 1988). Our recent study showed that single subcutaneous (s.c.) injection and oral administration of AITC (20 mg/kg body weight) significantly reduced the number of lymphocytes at 4 hr by approximately 32%, as compared with the control (Imaizumi et al., 2010). These results indicate that decreased effects of AITC on the number of lymphocytes are independent on the administration route. In the present study, therefore, AITC (dose=20 mg/kg body weight/day) was administered from the cervical portion of the back via a s.c. injection (9:00-9:30 a.m.) (Shirato et al., 2007). In the control group, an equivalent volume of AITC-free solution was administered to rats in the same manner.

Blood analyses and plasma preparations

![Fig. 1.](image-url) Experimental protocol. AITC (dose = 20 mg/kg body weight/day) was administered to rats from the cervical portion of the back via a subcutaneous (s.c.) injection (9:00-9:30 a.m.) for 10 days. The number of total WBCs, lymphocytes, monocyte, neutrophil, eosinophil and basophil during the experimental period were determined by flow cytometry. Plasma corticosterone concentrations during the experimental period were assayed after the experiment.
Effects of AITC on the number of WBCs

After the prefeedings for 5 days, the rats were randomly divided into the AITC group (dose = 20 mg/kg body weight/day, n = 12, the initial body weight = 268 ± 2g, mean ± S.E.) and the control group (n = 8, the initial body weight = 272 ± 3g, mean ± S.E.) on the final day of the adaptation. AITC was administered via s.c. injection at the dose of 20 mg/kg body weight/day to rats for consecutive 10 days. Blood samples were collected from tail vein on days 1, 2, 4, 6, 8 and 10 post s.c. injection (Fig. 1). The analyses of the number of WBCs (total WBCs, lymphocytes, monocyte, neutrophil, eosinophil and basophil) were carried out by the hematology analyzer (Model SF-3000, Sysmex Co., Kobe, Japan) based on flow cytometry technique with light-emitting diode (Shirato et al., 2006).

Effects of AITC on plasma corticosterone concentrations

Plasma corticosterone concentrations on days 1, 2, 4, 6, 8 and 10 post s.c. injection were 6.2 (p < 0.001), 5.6 (p < 0.001), 8.4 (p < 0.001), 8.0 (p < 0.001), 5.6 (p < 0.001) and 4.7 (p < 0.001) times markedly higher in the AITC group than in the control group, respectively (Fig. 3).

Effects of AITC on the relative weights of visceral organs

Administration of AITC decreased the relative weight of the thymus (mg) per body weight (g) by 32% (p < 0.05) (the AITC group: 1.00 ± 0.08 and the control group: 1.46 ± 0.16). The relative weight of spleen (mg) per body weight (g) showed no significant differences between two groups. On the contrary, administration of AITC increased the relative weight of adrenals (mg) per body weight (g) by 21% (p < 0.05) (the AITC group: 0.103 ± 0.005 and the control group: 0.085 ± 0.005).

DISCUSSION

AITC-induced changes of the number of total WBCs

The number of lymphocytes+monocyte+neutrophil accounts for approximately 98% of the number of total WBCs (Akimoto et al., 2009). In the present study, AITC markedly decreased the number of lymphocytes on days 1-10 and monocyte on days 1-8 post s.c. injection (Figs. 2B and C). On the contrary, AITC clearly increased the number of neutrophil on days 8-10 post s.c. injection (Fig. 2D). Therefore, no significant differences of the number of total WBCs between the AITC and control groups were observed on days 6-10 post s.c. injection, because the decreased number of lymphocytes and monocyte was counterbalanced by the increased number of neutrophil (Fig. 2A). These results indicate that the number and distribution of total WBCs depend largely upon the dynamic changes of the number of lymphocytes, monocyte and neutrophil. These findings are important for the complete understanding of the effects of AITC on the number of WBCs.
Fig. 2. Effects of AITC on the number of total WBCs (A), lymphocytes (B), monocyte (C), neutrophil (D), eosinophil (E) and basophil (F). Values: mean ± S.E. (n = 8-12/group). The number of WBCs was analyzed on 3 hr post s.c. injection. Open circle: the control group and closed circle: the AITC group. Statistics: *p < 0.05, **p < 0.01 and ***p < 0.001 (vs. the control group).
ic nerves (Iwasaki et al., 2008; Watanabe et al., 1988). Generally, adrenaline is known to increase the number of neutrophil (neutrophilia), whereas decrease the number of lymphocytes (lymphocytopenia) in blood (Wenish et al., 1996). These known phenomena may support the idea that AITC-induced release of adrenaline in the circulation would lead at least in part to neutrophilia as observed here in the AITC group. On the other hand, glucocorticoid is also known to induce the mobilization of neutrophil from the bone marrow into the circulating blood, inhibit apoptosis of circulating neutrophil and suppress the inflammatory consequences of neutrophil migration and activation (Goulding et al., 1998). In the present study, plasma corticosterone concentrations on days 1-10 post s.c. injection were markedly higher in the AITC group than in the control group (Fig. 3). Therefore, higher plasma corticosterone levels may be related to AITC-increased number of neutrophil.

As already described, AITC markedly decreased the circulating number of lymphocytes on days 1-10 post s.c. injection (Fig. 2B). These results accord with our previous findings (Imaizumi et al., 2010). Catecholamine is known to exert a powerful impact on the immune system by downregulation of proliferation and differentiation of lymphocytes and induce apoptosis of lymphocytes (Josefsson et al., 1996). On the other hand, Dhabhar et al. (1995) reported that infusion of the synthetic glucocorticoid into rats decreased lymphocyte numbers in blood accompanied by retention of circulating lymphocytes within bone marrow, spleen, and lymph nodes. Furthermore, stress-induced increases in plasma corticosterone are also shown to be accompanied by significant decreases in the number and percentage of lymphocytes (Dhabhar et al., 1995). It is possible that the decreased number of lymphocytes has inverse relationship with increased corticosterone concentrations. These results show that higher plasma corticosterone concentrations correlate inversely with the decreased number of lymphocytes (Figs. 2B and 3).

AITC is known to enhance sympathetic nervous activities and energy metabolism through TRPA1 which has an important role in the responses to endogenous inflammatory mediators and volatile irritants such as AITC and cinnamaldehyde (Imaizumi et al., 2010). Therefore, AITC-induced promotion of sympathetic nervous activities may increase markedly plasma corticosterone levels (Fig. 3). As shown in the present study, AITC-induced reduction of thymus mass would be partially caused by glucocorticoid-induced apoptosis cell death in thymocytes (Shirato et al., 2007; Someya et al., 2009). It is well known that glucocorticoids are adrenal steroid hormones with anti-inflammatory actions and induce immature T lymphocyte

![Fig. 3.](image-url) Effects of AITC on plasma corticosterone concentrations. Values: mean ± S.E. (n = 8-12/group). Plasma corticosterone concentrations during the experimental period were assayed on 1 hr post s.c. injection. Open circle: the control group and closed circle: the AITC group. Statistics: ***p < 0.001 (vs. the control group).
and thymus cell apoptosis (Ohkaru et al., 2010). On the contrary, the reactivity of peripheral lymphocytes in the spleen against AITC may be relatively lower than that of thymocyte (Imaizumi et al., 2010; Ohkaru et al., 2010). As already described, AITC-induced adrenal hypertrophy would be caused by stress-induced activation of the hypothalamo-hypophyseal-adrenocortical axis by catecholamines such as adrenaline and noradrenaline (Shirato et al., 2006; Shirato et al., 2007). Therefore, the physiological defense system via the endocrine system may be at least in part activated by AITC.

**AITC-induced changes of the number of monocyte, eosinophil and basophil**

Van Furth and Sluiter (1986) studied the distribution of monocyte in mice, and showed that the circulating monocyte account for 40% and marginated monocyte account for 60% of the peripheral blood monocyte. These findings suggest that the circulating number of monocyte is capable of being increased up to 2.5-times when all of the marginated monocytes are mobilized into the circulating blood. In the present study, AITC decreased the number of monocyte (Fig. 2C). On the contrary, AITC did not change the number of eosinophil and basophil (Figs. 2E and F). These results suggest that AITC and catecholamines may employ different mechanisms when they induce innate immune cells such as neutrophil, monocyte, eosinophil and basophil. Further systematic studies are also necessary, however, to clarify the mechanisms involved in the effects of AITC on the number of WBCs in rats.

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