Subacute Effects of Capsaicinoids on the Distribution of White Blood Cells in Rats

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The subacute effects of dihydrocapsaincin (DHC) and capsaicin (CAP) (dose = 3 mg/kg body weight per day for 10 days, subcutaueous) on the number and distribution of white blood cells (WBCs) were studied in male adult rats. The administration of DHC and CAP for 10 days decreased significantly the number of total WBCs, lymphocytes and monocyte, and increased significantly the number of the neutrophil and eosinophil without changing the number of basophil. The administration of DHC significantly decreased thymus weight and increased adrenal weight, showing that DHC induced thymus atrophy and adrenal hypertrophy. These results suggest that capsaicinoids induced the decrease of acquired immunity responses and these phenomena may have in part participated in capsaicinoids-induced stress-responses in rats.

Key words —— capsaicinoid, capsaicin, dihydrocapsaicin, white blood cell, lymphocyte

INTRODUCTION

Red chili peppers (*Capsicum frutescens*) have been used for several thousand years as food additives and for abroad variety of medical applications in Indian, Native American, African, and Chinese medical traditions.[1)] Red chili pepper is described to enhance immune response, act in an anti-inflammatory manner, lower blood pressure, reduce excessive blood clotting, reduce blood sugar level, although no formal examinations of these claims have been reported.[2)]

Two major capsaicinoids, dihydrocapsaicin (DHC) and capsaicin (CAP) are responsible for up to 90% of total pungency of red chili pepper fruits.[1)] DHC and CAP are typical capsaicinoids whose differences of chemical structures are absence or presence of double bond between the carbon atoms in alkyl side chain groups.[3)] Capsaicinoids are known to enhance energy metabolism through catecholamine secretion from adrenal medulla as a result of the activation of the central nervous system and which was mediated through thermosensitive transient receptor potential (TRP) channels V1 (TRPV1).[4, 5)] Activation of TRPV1 plays a role not only in transmission of the pungent or pain sensations but also enhancement of CAP-induced energy consumption and thermogenesis.[6)]

The number and distribution of white blood cells on immune responses provide an important representation of the state of activation of the immune system.[7)] Capsaicinoids are also known to effect immune-response system by various factors such as neuropeptides and adrenal hormones,[7)] and to induce different physiological responses between DHC and CAP.[8)] However, there is very little scientific evidence for chronic effects of capsaisinoids on immuno-response properties.[1)] Recently, we reported that a single administration [dose = 3 mg/kg body weight (BW)] of capsaicinoids such as DHC and CAP to rats decreased significantly the number of total lymphocytes, T-lymphocyte and B-lymphocyte without changing the number of natural killer (NK) cells.[8)] However, the subacute and chronic effects of capsaicinoids on the number and distribution of white blood cells (WBCs) are still unknown. In the present study, we studied the subacute effects of DHC and CAP on the number of total WBCs, lymphocytes, monocyte, neutrophil, eosinophil and basophil in rats. The subacute effects of capsaicinoids on the weight of thymus, spleen and adrenals were also examined.

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METHODS AND MATERIALS

Experimental Procedures and Animal Care —— The experimental protocol used in the present study is shown in Fig. 1. Male 6-week-old Sprague Dawley rats (CLEA Japan, Tokyo, Japan) were pre-fed for 4 days to allow adaptation to their new environment.[8–13] After the adaptation period, the rats were randomly divided into three groups, DHC (initial BW = 234 ± 2 g, in mean ± standard error of the mean), CAP (initial BW = 233 ± 3 g) and the control (CON: initial BW = 233 ± 2 g) groups. DHC and CAP (each dose = 3.0 mg/kg BW per day) were administered to rats for 10 days.[8] The numbers of total WBCs, lymphocytes, monocyte, neutrophil, eosinophil and basophil were assayed. Thymus, spleen and adrenals were isolated and weighed. All experimental and animal care procedures were approved by the Committee on Animal Care and Use of Waseda University. This study was performed with least possible pain or discomfort to the rats.[8–13]

Administrations of DHC and CAP to Rats —— DHC and CAP (Sigma, St. Louis, MO, U.S.A.) were prepared in 2% ethanol containing 2% Tween 80 and 0.9% NaCl solution as a vehicle to obtain 0.1% solution.[8] DHC (purity ≥ 95%) and CAP (purity ≥ 90%) were administered to rats via subcutaneous (s.c.) injection from cervical portion of the back for 10 days (9:00–10:00, a.m.).[8] An equivalent volume of capsaicinoids-free solution was administered to CON group rats.[8]

Blood Samplings and Count Analyses of WBCs —— Fifty microliters blood samples were collected with heparinized microcapillary tubes (Tokyo Glass Co., Tokyo, Japan) from tail vein at 3 hr after the administration,[8] and the number of WBCs were analyzed with the hematology analyzer (Model SF-3000, Sysmex Co., Hyogo, Japan).[8, 11–13]

Statistical Analyses —— Experimental data were presented as means ± SEM. Data were tested by a one-way analysis of variance (ANOVA) and a two-way ANOVA for repeated measures. The differences were considered significant when p was < 0.05.

RESULTS AND DISCUSSION

The Body Weight, Body Weight Gain, Food Intake, Water Intake and Food Efficiency during the Experimental Period

The present study clearly showed that the administration of DHC for 10 days decreased significantly the body weight, body weight gain and food efficiency (= total food intake/ΔBW) without changing total food intake. However, the administration of CAP decreased significantly only the body weight gain. The magnitude of the effects of capsaicinoids on these parameters was relatively higher in DHC group than in CAP group (Table 1).

The Absolute Weight of Thymus, Spleen and Adrenals

The present study showed that the administration of DHC decreased significantly the absolute weight of thymus, one of the central lymphoid organs, to 0.82 times and increased the absolute weight of adrenals to 1.14 times, as compared with CON group (Fig. 2). However, no differences in the absolute weight of spleen, one of the peripheral lymphoid organs, were observed in three groups (Fig. 2).

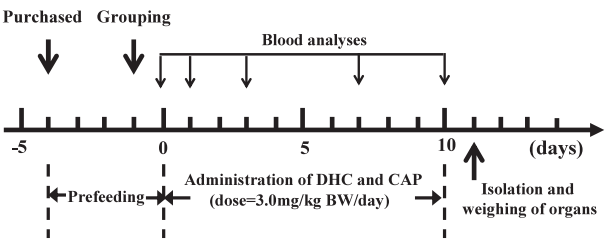

Fig. 1. Experimental Protocol Used in the Present Study

Down-arrow: Blood sampling, flow-cytometrical analyses of the number of total WBCs, lymphocytes, monocyte, neutrophil, eosinophil and basophil, and hematocrit. Up-arrow: Thymus, spleen and adrenals were isolated, and weighed on the next day of final administration.

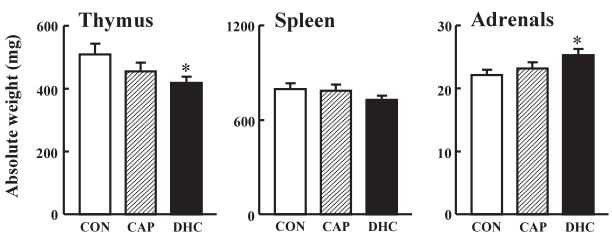

Fig. 2. Subacute Effects of DHC and CAP on the Absolute Weight of Thymus, Spleen and Adrenals

Values: means ± SEM. □: CON group (n = 8), ▨: CAP group (n = 9) and ■: DHC group (n = 12). Statistics: $^{*}p < 0.05$ (*vs.* CON group).

Table 1. The Subacute Effects of Capsaicinoids on the Body Weight, Body Weight Gain, Food Intake, Water Intake and Food Efficiency during the Experimental Period

Parameters	Group					
	CON	DHC		CAP		
Body weight (g)	302 ± 2	290 ± 3	(0.96)**	294 ± 4	(0.97)	
Body weight gain (g/10 days)	70 ± 2	56 ± 2	(0.81)***	62 ± 3	(0.88)*	
Food intake (g/day)	25.5 ± 0.4	25.2 ± 0.5	(0.99)	24.6 ± 0.5	(0.97)	
Water intake (g/day)	46.4 ± 2.4	51.9 ± 2.5	(1.12)#	44.0 ± 2.5	(0.95)	
Food efficiency[a)]	0.27 ± 0.01	0.22 ± 0.01	(1.22)***	0.25 ± 0.01	(1.13)	

Values: means ± SEM. DHC: dihydrocapsaicin (dose = 3.0 mg/kg BW per day) group (n = 12), CAP: capsaicin (dose = 3.0 mg/kg BW per day) group (n = 9) and CON: control group (n = 8). *a*) Food efficiencies were calculated from the ratio of the body weight gain to total food intake during experimental period. Values in parentheses are shown as the relative ratio of DHC group or CAP group to CON group. Statistics: $^{*}p < 0.05$, $^{**}p < 0.01$ and $^{***}p < 0.001$ (*vs*. CON group), and $^{\#}p < 0.05$ (*vs*. CAP group).

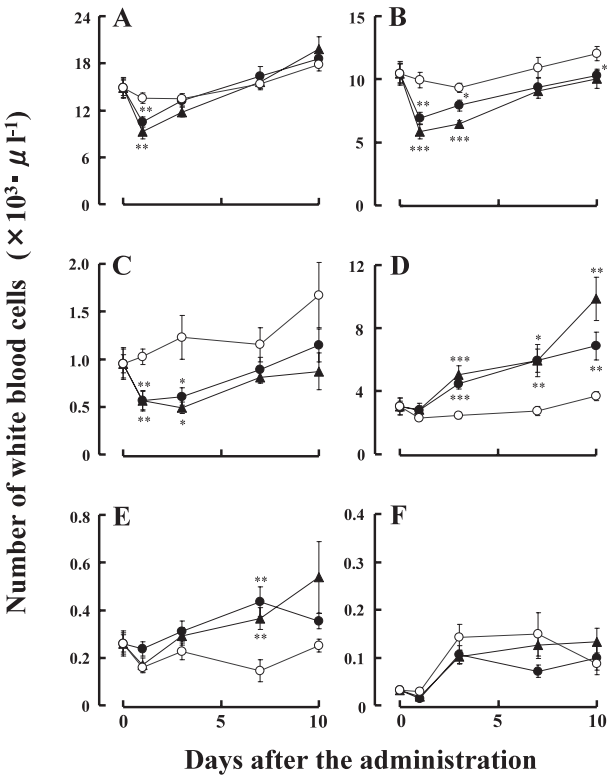

Fig. 3. Subacute Effects of DHC and CAP on the Number of WBCs during the Experimental Period

Values: means ± SEM. A: total WBCs, B: lymphocytes, C: monocyte, D: neutrophil, E, eosinophil, and F: basophil. ○: CON group (n = 8) and ●: CAP group (n = 9) and ▲: DHC group (n = 12). Statistics: $^{*}p < 0.05$, $^{**}p < 0.01$ and $^{***}p < 0.001$ (*vs*. CON group).

The Number of White Blood Cells

The numbers of total WBCs were 0.7–0.8 times significantly lower at 1 day in DHC and CAP groups than in CON group (Fig. 3A). The numbers of lymphocytes were 0.6–0.7 times significantly lower at 1–3 days in DHC group, and 0.7–0.9 times significantly lower at 1–10 days in CAP group than in CON group (Fig. 3B). The numbers of monocyte were also 0.4–0.6 times significantly lower at 1–3 days in DHC and CAP groups than in CON group (Fig. 3C). On the contrary, the administration of DHC increased significantly the number of neutrophil to 2.0–2.6 times at 3–10 days (Fig. 3D). The administration of CAP increased also significantly the number of neutrophil to 1.8–2.1 times at 3–10 days, as compared with CON group (Fig. 3D). The numbers of eosinophil were 1.4–2.9 times significantly higher at 7–10 days in DHC and CAP groups than in CON group (Fig. 3E). However, no significant differences among three groups were observed in the number of basophil (Fig. 3F).

Our preliminary results showed that the oral administration and subcutaneous injection of capsaicinoids to rats decreased significantly the number of lymphocytes at 2–4 days after the administration (data not shown). Further, we also observed that the oral administration and subcutaneous injection of allyl isothiocyanate (AITC), one of the main pungent components of Wasabi, to rats decreased significantly the number of lymphocytes at 4 hr after the administration.[14] These results suggest that capsaicinoids-induced reductions of the number of lymphocyte are independent on the administration routes of pungent components and depend mostly on capsaicinoids themselves.

The present study clearly showed that the administration of DHC and CAP decreased markedly the number of lymphocytes and monocyte at 1–3 days (Fig. 3B and C), and increased markedly the number of neutrophil at 3–10 days (Fig. 3D). The total numbers of lymphocytes, monocyte and neutrophil account for approximately 98% of the number of total WBCs.[8] Therefore, the number of total WBCs in DHC group cancelled out for the number of decreased lymphocytes and monocyte, and

increased neutrophil, and no significant differences at 3–10 days were observed as compared with CON group (Fig. 3A). 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.

The present study also showed that the administration of DHC and CAP decreased markedly the circulating number of lymphocytes at 1–3 days after the administration (Fig. 3B). Catecholamine is known to exert a powerful impact on the immune system by down-regulation of proliferation and differentiation of lymphocytes and induce apoptosis of lymphocytes.[15] This decreased number of lymphocyte (lymphocytopenia) may be associated with catecholamine-induced apoptosis of lymphocyte. However, the number of lymphocytes in DHC and CAP groups recovered to that in CON group at 7–10 days (Fig. 3B). These results suggest that these responses are reversible or redistributional phenomena.

On the other hand, Dhabhar *et al.*[7] reported that the infusion of the synthetic glucocorticoid into rats decreased the numbers of lymphocytes 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.[7] It is possible that the decreased number of lymphocytes has inverse relationship with increased corticosterone concentrations. These results show that serum corticosterone concentrations correlate inversely with the decreased number of lymphocytes. However, serum corticosterone concentrations at 3 hr after single administration of DHC and CAP did not significantly change, as compared with CON group.[8] From these findings, therefore, it is uncleared that serum coricosterone concentrations affect the decreased number of lymphocytes at 3 hr after the administration of capsaicinoids.[8] On the contrary, Cox[16] reported that glucocrticoid treatment inhibits apoptosis in human neutrophils. In the present study, DHC and CAP significantly increased the number of neutrophil, suggesting that DHC and CAP-induced increase of serum corticosterone concentrations upregulates the number of neutrophil.

Furthermore, Dhabhar *et al.*[7] showed that B lymphocyte is more sensitive to adrenal hormones than T lymphocyte, and NK cells are relatively less affected in terms of glucocorticoid-induced decreases in cell number in the blood. Our previous study showed that the acute effects of DHC and CAP on the number of T lymphocyte and B lymphocyte were relatively more affected in DHC than in CAP.[8] From these results, the acute effects of DHC on T lymphocyte and B lymphocyte show clearly more sensitive responses than those of CAP, and the suppressive effects of DHC on the number of B lymphocyte are also the most effective in the number of lymphocyte subsets.[8]

In the present study, DHC and CAP decreased markedly the number of monocyte (Fig. 3C). Shirato *et al.*[11] reported that β_2-adrenergic agonist, clenbuterol increased the circulating number of monocyte, suggesting that clenbuterol-induced monocytosis might be mediated mainly by the mobilization from the vessel margin. In the present study, however, DHC and CAP did not induce monocytosis (Fig. 3C). On the contrary, DHC and CAP increased the number of eosinophil at 7–10 days (Fig. 3E). In addition, the number of basophil did not also change significantly by the administration of DHC and CAP (Fig. 3F). Van Furth and Sluiter[17] studied the distribution of monocyte in mice, and reported 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 conclusion, the administration of capsaicinoids (DHC and CAP) induced the decrease of acquired immunity responses and these phenomena may have in part participated in capsaicinoids-induced stress-responses in rats.

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