**Acute Effects of Dihydrocapsaicin and Capsaicin on the Distribution of White Blood Cells in Rats**

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**Summary**

The acute effects of dihydrocapsaicin (DHC) and capsaicin (CAP) on the number of white blood cells (WBCs), neutrophils, eosinophils, basophils, monocytes, lymphocytes, T lymphocytes, B lymphocytes and NK cells, and serum corticosterone levels were studied in rats. Male 7-wk-old SD rats were divided into DHC (3.0 mg/kg BW), CAP (3.0 mg/kg BW) and control (CON) groups. The number of total WBCs was 1.30–1.42 times significantly higher in the DHC group than in the CON group at 6–12 h. The number of neutrophils was 1.62 times significantly higher in the DHC group than in the CON group at 12 h. The number of total WBCs and neutrophils, however, showed no significant changes between the CAP and CON groups. The number of lymphocytes was 0.61 and 0.70 times significantly lower in the DHC and CAP groups than in the CON group at 3 h. The number of T lymphocytes and B lymphocytes was 0.74 and 0.54 times lower in the DHC group than in the CON group, respectively. CAP, however, did not significantly change the number of T lymphocytes or B lymphocytes. No significant changes in the number of NK cells were observed among the three groups. CAP and DHC did not change the number of monocytes, eosinophils or basophils. No significant changes of the serum corticosterone levels were observed among the three groups. In conclusion, capsaicinoids decreased the number of acquired immunity cells, and increased the number of total WBCs and neutrophils without changing the number of monocytes, eosinophils or basophils. The magnitude of these effects was relatively higher in DHC than in CAP.

**Key Words**
capsaicinoids, capsaicin, dihydrocapsaicin, white blood cells, lymphocytes

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Hot peppers are used as a spice for enhancing the palatability of food and drugs such as counterirritant stomach medicines in many countries. The pungent principle of hot pepper is a group of compounds called capsaicinoids, which possess a variety of biological properties, and capsaicinoids are a family of natural products isolated from the dried fruit of chili peppers (1). These substances are the principals those produce the characteristic sensations with the ingestion of spicy cuisine as well as the agent responsible for cuisine (2, 3). The two major capsaicinoids, capsaicin (CAP) and dihydrocapsaicin (DHC) are responsible for up to 90% of the total pungency of pepper fruits (2). DHC and CAP are typical capsaicinoids whose differences of chemical structures are the presence or absence of a double bond between the carbon atoms in alkyl side chain groups (1–3).

Capsaicinoids have been reported to enhance energy metabolism through catecholamine secretion from the adrenal medulla as a result of the activation of the central nervous system and which was mediated through thermosensitive transient receptor potential (TRP) channels, A1 (TRPA1) and V1 (TRPV1) (4). The TRPV1 is activated by volatile pungent foods such as hot pepper (capsaicine), black and white pepper (piperine) and ginger (gingerol). Further, low or high temperatures also effect TRPV1 (<18˚C), and TRPV1 (>43˚C). Therefore, they are called thermosensitive TRP channels (4–6). Activation of TRPA1 plays a role not only in transmission of the pungent or pain sensations but also enhancement of CAP-induced energy consumption and thermogenesis. CAP enhances energy metabolism via adrenalin secretion from the adrenal medulla through activation of the central nervous system (3–7). In addi-
tion, the effects of capsaicinoids on body heat production, lipid and energy metabolism, swimming endurance capacity, antioxidant activity and perspiration have been reported by many studies (1–6).

Gamse et al. (8) reported that CAP acutely releases immuno-reactive substance P (SP) from terminals of primary afferents in the spinal cord. SP released from primary afferent neurons of rats by capsaicinoids is known to play an important role in the regulation of several immune responses, and both in vivo and in vitro studies have indicated that SP stimulates macrophage phagocytosis (9). It is also reported that capsaicin can influence the secretion of other immunoreactive neuropeptides, which are associated with immune responses (10), and has been shown to have immunomodulatory effects, as indicated by its ability to modulate lymphocyte proliferation and immunoglobulin production (11). These results suggest that CAP affects immune systems directly or indirectly through various actions. In addition, DHC is known to play a role similar to CAP in biological actions, but has somewhat greater potency and is more effective in producing hypothermia than CAP (12). These phenomena suggest that DHC has strong effects in physiological functions as compared with CAP.

It is well known that the number and distribution of white blood cells on immune responses provide an important representation of the state of activation of the immune system (13). Capsaicinoids are also known to affect immune-response systems by various factors such as neuropeptides and adrenal hormones (13), and different physiological responses are known to be induced between DHC and CAP. However, the acute and chronic effects of capsaicinoids such as DHC and CAP on the number and distribution of white blood cells are still unknown. In the present study, therefore, the acute effects of DHC and CAP on the number of white blood cells, neutrophils, lymphocytes, T lymphocytes, B lymphocytes and NK cells, monocytes, eosinophils, basophils, and serum corticosterone concentrations were studied in rats. The effects of DHC and CAP on the number and distribution of white blood cells were also compared between DHC and CAP.

**Methods and Materials**

Two parts of an experiment. 1) the acute effects of dihydrocapsaicin (DHC) and capsaicin (CAP) on the number of total white blood cells (WBCs), neutrophils, lymphocytes, monocytes, eosinophils and basophils, and 2) the acute effects of DHC and CAP on the numbers of T lymphocytes, B lymphocytes and NK cells, and serum corticosterone concentrations were studied in rats. Male 7-wk-old Sprague-Dawley rats (CLEA Japan, Inc., Tokyo) were prefed for 5 d to allow adaptation to their new environment. The rats were housed in cages at a temperature of 22–25°C and a relative humidity of 50–60% (14, 15). Lighting was automatically provided from 8:00 to 20:00 (14, 15). Animal chow (CE-2, CLEA Japan, Inc.) and once-boiled tap water were given to the rats ad libitum (16). After the adaptation period, the rats were randomly divided into three (DHC, CAP and control (CON)) groups. The present study was carried out according to the “Guiding Principle for the Care and Use of Animals in the Field of Physiological Sciences” of the Physiological Society of Japan (17). These experimental protocols were also approved by the Animal Ethics Committee, Faculty of Human Sciences, Waseda University. We caused the least possible pain or discomfort to the rats.

**Administrations of DHC and CAP to rats.** DHC and CAP (Sigma, St Louis, MO) were dissolved in 2% ethanol, and then supplemented with 10% Tween 80 and 0.9% NaCl solution as a vehicle to obtain a DHC or CAP concentration of 0.1%. DHC and CAP were administered as dosage of 3.0 mg/kg BW via subcutaneous (sc) injection from the cervical region of the back (15, 18). An equivalent volume of capsaicinoid-free solution was administered to CON group rats in the same manner.

**Count analyses of white blood cells.** The number of WBCs was analyzed at constant intervals after the administration of DHC, CAP and CON. Count analyses of WBCs were carried out with a hematology analyzer (Model SF-3000, Sysmex Co, Hyogo) based on a flow cytometry technique with a light-emitting diode (18–20).

**Blood samplings and animal treatments.** Whole blood samples (60–70 μL) were collected from the tail vein according to our routine method (18–20). These samples were used for count analyses of WBCs (18–20). At 3 h after the administration, the animals were anesthetized with sodium pentobarbital (50 mg/kg BW), and then blood samples were collected for the assays of the serum corticosterone concentrations and lymphocyte subsets. Blood for the assay of serum corticosterone was collected from the abdominal aorta in a vacuum tube with serum separating agent for collecting blood at 3 h after the administration.

Blood samples for analysis of T lymphocytes, B lymphocytes and NK cells were also collected from the abdominal aorta in a heparinized vacuum tube with an anticoagulant at 3 h (20).

**Analyses of lymphocyte subsets and assay of serum corticosterone concentration.** T lymphocytes, B lymphocytes and NK cells were analyzed by our routine method (20). The assay of serum corticosterone concentration was carried out by using the Active Rat Corticosterone EIA kit (DSL, Inc, USA) with a microplate reader (DTX 800/880 Multimode Detectors, Beckman Coulter, USA).

**Statistical analyses.** Experimental data were presented as means ± standard error of the mean (SE). The effects of DHC and CAP on the number of WBCs were analyzed by two-way ANOVA with repeated measures, and statistical significance (p<0.05) among the three groups was estimated using Tukey-Kramer multiple comparison tests. The effects of DHC and CAP on the number of T lymphocytes, B lymphocytes and NK cells, and serum corticosterone concentrations were analyzed by one way ANOVA, and then by using Tukey-Kramer multiple comparison tests.
**Results**

**Effects of DHC and CAP on the number of RBCs, hematocrit value, hemoglobin concentration, MCV, MCH and MCHC values**

Although data are not shown, there were no significant changes in the number of red blood cells (RBCs), hematocrit value, hemoglobin concentration, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) or mean corpuscular hemoglobin concentration (MCHC) in 3–24 h after administration among the three groups. These results suggest that the single administration of DHC and CAP to rats did not change extracellular fluid volume or hematopoietic capacities.

**Effects of DHC and CAP on the number of total WBCs and neutrophils**

As shown in Fig. 1A, the number of total WBCs in the DHC group was 1.30 ($p<0.05$), 1.30 ($p<0.05$) and 1.42 times ($p<0.05$) higher than that in the CON group at 6, 9 and 12 h after the administration of DHC, respectively. However, no significant differences between the CAP group and the CON group in the number of total WBCs were observed. The number of neutrophils in the DHC group was 1.62 times ($p<0.05$) higher than that in the CON group at 12 h (Fig. 1B). Although the number of neutrophils in the CAP group was relatively higher than that in the CON group at 9 and 12 h, no significant changes between the CAP group and the CON group were observed. The number of lymphocytes in the DHC and CAP groups was 0.67 ($p<0.05$) and 0.77 times ($p<0.01$) lower than that in the CON group at 12 h (Fig. 1C). However, the number of lymphocytes in the DHC and CAP groups at 6 h recovered nearly to the level of the CON group (Fig. 1C). Although data are not shown, DHC or CAP did not change the number of eosinophils, basophils or monocytes.

**Effects of DHC and CAP on the number of lymphocytes, T lymphocytes, B lymphocytes and NK cells**

Figure 2 shows the number of lymphocytes, T lymphocytes, B lymphocytes and NK cells in the three groups at 3 h. The number of lymphocytes in the DHC and CAP groups was 0.67 ($p<0.05$) and 0.77 times lower than in the CON group, respectively (Fig. 2A). The number of T lymphocytes in the DHC group was 0.74 times ($p<0.05$) clearly lower than that in the CON group (Fig. 2B). However, the number of T lymphocytes in the CAP and CON groups was not significantly changed (Fig. 2B). Although the number of B lymphocytes in the DHC group was 0.54 times ($p<0.05$) lower than that in the CON group (Fig. 2C), no significant differences in the number of B lymphocytes between the CAP and CON groups were observed (Fig. 2C). On the other hand, no significant changes in the number of NK cells were observed among the three groups (Fig. 2D).

**Effects of DHC and CAP on serum corticosterone concentrations**

No significant changes of corticosterone concentrations at 3 h after the administration were observed.
among the three groups (DHC group: 1.93 ± 0.08 μg/mL, CAP group: 1.92 ± 0.05 μg/mL, CON group: 1.79 ± 0.04 μg/mL in means ± SE (n = 6–7/group)).

### Discussion

The main findings of the present study are summarized in Table 1 as follows: DHC (Fig. 1) increased markedly the number of total WBCs (Fig. 1A) and neutrophils (Fig. 1B) at 6–12 h, but significantly decreased the number of lymphocytes (Fig. 1C), and T lymphocytes and B lymphocytes at 3 h (Fig. 2B and C). Although similar responses were also observed by the administration of CAP (Figs. 1 and 2), the magnitude of the acute effect of CAP on total WBCs, neutrophils and lymphocytes was relatively smaller than those of DHC (Table 1). These results suggest that DHC and CAP markedly decrease the number of acquired immune cells such as lymphocytes and these subsets early on, but the change of the number in the DHC and CAP groups on their immuno-responsible cells is a reversible phenomenon because the number of lymphocytes in DHC and CAP groups recovered to the baseline number (Fig. 1). DHC and CAP increased innate immune cells such as neutrophils with the increase of the time (Fig. 1B), and the response pattern of the number of neutrophils during the experiment was relatively slow and long as compared with the changes of lymphocytes (Fig. 1B). However, other innate immune cells such as basophils, eosinophils and monocytes were not significantly changed by DHC or CAP. Further, the magnitudes of the effects of capsaicinoids on the number of total WBCs were greater in DHC than in CAP (Table 1).

### Acute effects of DHC and CAP on the number of total WBCs

It is generally accepted that the number of neutrophils + lymphocytes accounts for approximately 90% of the number of total WBCs. In the present study, the number of lymphocytes in the DHC group decreased markedly at 3 h (Figs. 1C and 2A). On the other hand, the number of neutrophils in DHC group relatively increased at 3 h (Fig. 1B). Therefore, the number of total WBCs in DHC groups cancelled out for the number of decreased lymphocytes and increased neutrophils, and no significant differences were observed as compared with the CON group. However, after this, the number of lymphocytes in the DHC group recovered to the levels of the CON group at 6 h and the number of neutrophils in the DHC group continued to increase until 6 h (Fig. 1B). Because of the recovery of the number of lymphocytes and the increased number of neutrophils at 6 h, the number of total WBCs in the DHC group was significantly higher compared with the CON group. These responses were also found until 9–12 h (Fig. 1A). Although similar responses were found in the CAP group in 3–12 h, no significant differences were observed as compared with data for the CON group at any time. These results indicate that the number and distribution of total WBCs depend largely upon the dynamic changes of the number of neutrophils and lymphocytes.

### Acute effects of CAP and DHC on the number of neutrophils and lymphocytes

It is reported that an intravenous injection of CAP caused a significant increase in adrenal sympathetic efferent nerve activity, and CAP-induced adrenal catecholamine secretion was elicited through activation of the adrenal sympathetic nerves (21). Generally, adrenaline is known to increase the number of neutrophils (neutrophilia), whereas the number of lymphocytes is decreased (lymphocytopenia) in blood (22). This neutrophilia by catecholamine has been found to be mobilized from the marginated pool into the circulation by the arterial shearing force through β-adrenergic enhancement of heart functions, because adrenalin binds with β-receptors (23). From these phenomena, the increasing adrenaline in circulation would lead to the neutrophilia of the DHC group. In fact, prolonged inactivity [hypokinesia (i.e. decreased motor activity)/hypodynamia (i.e. decreased mechanical loading)] conditions and β2-agonist clenbuterol increased significantly the number of neutrophils (18–20). In the present study, although it was not investigated whether synthesis of the hematopoietic cytokine colony-stimulated factor (G-CST) or macrophage colony-stimulating factor (M-CSF) are induced by prolonged inactivity, at
least stress hormones such as catecholamines and glucocorticoids are likely involved in the neutrophils due to stress responsive changes of visceral organ weights (18–20).

On the other hand, glucocorticoid (corticosterone) is known to induce the mobilization of neutrophils from the bone marrow into the circulating blood (24), inhibit apoptosis of circulating neutrophils (25) and suppress the inflammatory consequences of neutrophil migration and activation (26). In the present study, however, serum corticosterone concentrations in the DHC and CAP groups at 3 h did not change significantly as compared with the CON group. Therefore, these results suggest that factors other than serum coricosterone affect neutrophilia. These possibilities need further elaborate studies.

As shown in the present paper, DHC and CAP markedly decreased the circulating number of lymphocytes at 3 h (Fig. 1C). Catecholamine is known to exert a powerful impact on the immune system by downregulation of proliferation and differentiation of lymphocytes (27) and to induce apoptosis of lymphocytes (28). This decreased number of lymphocytes (lymphocytopenia) would be caused by apoptosis of lymphocytes induced by catecholamine. However, the number of lymphocytes in the CAP and DHC groups recovered to that in the CON group at 6 h (Fig. 1C). Therefore, these responses are reversible or redistributal phenomena.

On the other hand, it is reported that infusion of the synthetic glucocorticoid into rats results in a decrease in lymphocyte numbers in the blood, that is accompanied by retention of circulating lymphocytes within bone marrow, spleen, and lymph nodes (29). Furthermore, stress-induced increases in plasma corticosterone are also shown to be accompanied by significant decreases in numbers and percentages of lymphocytes (29). It is possible that the decreased number of lymphocytes has an inverse relationship with increased corticosterone concentrations. However, serum corticosterone concentrations in the DHC and CAP groups at 3 h did not significantly change as compared with the CON group. From these findings, therefore, it is unclear whether serum corticosterone concentrations affect the decreased number of lymphocytes at 3 h.

Furthermore, Dhabhar et al. (29) 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 numbers in the blood. In the present study, the number of T lymphocytes and B lymphocytes were relatively more affected in the DHC than in the CAP group (Fig. 2B and C). On the other hand, the number of NK cells in the DHC and CAP groups was relatively lower than in the CON group, but no significant changes were observed among the three groups (Fig. 2D). From these results, the effects of DHC on T lymphocytes and B lymphocytes show more sensitive responses than those of CAP, and the suppressive effects of DHC on the number of B lymphocytes are the most effective in the number of lymphocyte subsets (Fig. 2B–D).

**Acute effects of CAP and DHC on the number of monocytes, eosinophils and basophils**

Van Furth and Sluiter (30) studied the distribution of monocytes in mice and reported that circulating monocytes account for 40% and margined monocyte account for 60% of the peripheral blood monocytes. These findings suggest that the circulating number of monocytes is capable of being increased up to 2.5 times when all of the margined monocytes are mobilized into the circulating blood. In the present study, the number of monocytes in the three groups showed no significant changes (data not shown). Shirato et al. (18) reported that the circulating number of monocytes was increased by the administration of β-adrenergic agonist (clenbuterol), suggesting that clenbuterol-induced monocytopoiesis might be mediated mainly by the mobilization from the vessel margin. In the present study, however, DHC and CAP did not induce monocytopoiesis. In addition, the number of eosinophils and basophils were not changed significantly by CAP or DHC, either. On the other hand, β-adrenergic stimuli are reported to induce the removals of eosinophils and basophils from the circulating blood (18). Although β-adrenergic stimuli are known to induce monocytopoiesis and the decreased number of eosinophils and basophils in blood, CAP and DHC did not affect the number of monocytes, eosinophils or basophils. The responses of these innate immune cells such as neutrophils, monocytes, eosinophils and basophils induced by CAP and DHC may be induced via different mechanisms from catecholamine. Further studies are indispensable to clarify the effects of oral administration of capsaicinoids on the number of white blood cells and lymphocyte subsets (T lymphocytes, B lymphocytes and NK cells) in rats.

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