Note

Stimulation of Tumor Necrosis Factor and Interleukin-1 Productivity by the Oral Administration of Cabbage Juice to Rats

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The effect of orally administering cabbage juice on tumor necrosis factor α (TNF) and interleukin-1 (IL-1) productivity was studied in resident peritoneal macrophages from normal and hepatoma-bearing rats. The productivity of TNF and IL-1 was stimulated by gastric intubation of cabbage juice in the normal state, but not in the hepatoma-bearing state where the production of these cytokines had already been stimulated. From these results, cabbage may contain some effective component(s) that can be absorbed from the gastrointestinal tract to stimulate the production of TNF and IL-1.

Key words: cabbage juice; tumor necrosis factor; interleukin-1; hepatoma

Tumor necrosis factor α (TNF) and interleukin-1 (IL-1) are the primary cytokines that are mainly produced by activated macrophages. TNF has been recognized as a serum factor to induce the hemorrhagic necrosis of a transplanted murine tumor, and plays important roles in antitumoral, antiviral, immunoregulatory and inflammatory responses. Moreover, TNF and IL-1 are known to share several biological roles.

There are two steps in the process of producing TNF: a priming step (e.g., a cytokine or biological response modifier pretreatment) and a triggering step (e.g., a bacterial or lipopolysaccharide treatment). Yamazaki et al. have recently reported that various vegetable juices, when intravenously administered to mice, had a strong priming effect, increasing the endogenous production of TNF following an OK-432 challenge. We have therefore attempted in this study to examine whether the oral administration of cabbage juice would be capable of affecting the productivity of both TNF and IL-1 by resident peritoneal macrophages in normal and hepatoma-bearing rats.

Male Donryu rats (4 wk of age, NRC Haruna, Gunma, Japan) were kept on a stock pellet diet (CE-2; CLEA Japan, Tokyo, Japan) for 4 d and then on a 20% casein diet containing 5% corn oil for another 7 (experiment 1) or 10 (experiment 2) days in an air-conditioned room with an 8:00 a.m. to 8:00 p.m. light cycle. A cabbage was obtained from a local market in Fuchu, Tokyo, and cabbage juice was prepared from washed cabbage and an equal weight of distilled water in a mixer, the contents being homogenized and centrifuged. The supernatant was filtered throughToy No. 2 filter paper (Advantec Toyo Co., Tokyo, Japan). In experiment 2, the rats received an s.c. implantation of $5 \times 10^8$ AH109A cells (an ascites hepatoma cell line provided by SRL, Tokyo, Japan) suspended in phosphate-buffered saline to produce a solid tumor in the back, as described previously. In both experiments, the rats were divided into two groups of similar body weights, and one group was orally given cabbage juice (1 ml/100 g of body weight/day) and the other group, regarded as the control, was orally administered with distilled water (1 ml/100 g of body weight/day) for 14 d. The oral administration was carried out once a day at 9:00-10:00 a.m. The rats were deprived of their diets at 9:00 a.m. on the scheduled days, but allowed free access to water until their decapitation 4 h later. Resident peritoneal macrophages were harvested by lavage from the peritoneal cavity with RPMI 1640 medium (Nissui Pharmaceutical Co., Tokyo, Japan), which had been prepared in endotoxin-free water for injection (Otsuka Pharmaceutical Co., Tokushima, Japan), containing 100 units/ml of penicillin G (Banyu Pharmaceutical Co., Tokyo, Japan), 100 μg/ml of streptomycin (Meiji Seika Kaisha, Tokyo, Japan), 2 mM L-glutamine (Wako Pure Chemical Industries, Osaka, Japan) and 50 μM 2-mercaptoethanol (Sigma Chemical Co., St. Louis, MO, U.S.A.), as described previously. The cells were washed twice with RPMI 1640 at 4°C, resuspended in RPMI 1640 supplemented with 10% fetal bovine serum (FBS, Irvine Scientific, Santa Ana, CA, U.S.A.; 10% FBS/RPMI 1640), seeded at 1 x 10⁶ cells/well into a 6-well flat-bottom plate (Corning Glass Works, Corning, NY, U.S.A.) and incubated at 37°C in an atmosphere of 5% CO₂/95% humidified air. After 2 h, the nonadherent cells were removed by washing three times with RPMI 1640, and the adherent cells were incubated for another 3 h in the presence of 1 μg/ml of lipopolysaccharide (LPS, from Salmonella typhimurium, obtained from Sigma Chemical Co.). At the end of the incubation, the culture supernatant was collected and frozen at −20°C until needed for analysis. The cells were washed once with RPMI 1640, dissolved in 0.1% sodium dodecyl sulfate (Wako Pure Chemical Industries), and their DNA contents were measured according to the procedure of Brunk et al. TNF and IL-1 activities in the culture supernatant were measured as described previously, using L929 mouse fibroblasts (obtained from RIKEN Cell Bank, Tsukuba, Japan) and D10.G4.1 mouse helper T cell clones (obtained from the American Type Culture Collection, Rockville, MD, U.S.A.), respectively. Each result is expressed as the mean ± SEM. A statistical analysis was carried out by one-way analysis of variance; when the F value was significant ($p < 0.05$), the difference was inspected by Student’s t-test.

In experiment 1, the effect of orally administering cabbage juice was first investigated in normal rats. The cabbage juice administration did not affect the food intake or body weight gain (data not shown). The production of TNF and IL-1 by resident peritoneal macrophages is illustrated in the Fig. TNF and IL-1 production was significantly enhanced by the oral administration of cabbage juice, the enhancement in the cabbage group compared with the control group being 180% for TNF and 156% for IL-1. Next, the productivity of the cytokines was examined in AH109A-bearing rats in experiment 2. The production of TNF and IL-1, however, showed no significant difference between the control and cabbage groups (Table).

The present study investigated the effect of an oral administration of cabbage juice on TNF and IL-1 productivity by resident peritoneal macrophages in normal and hepatoma-bearing rats, and demonstrated the elevation of TNF and IL-1 productivity in cabbage-treated, normal rats. These results indicate that cabbage

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has factor(s) which stimulate TNF and IL-1 production in macrophages and that the factor(s) can act by oral administration as well as by intravenous injection. Although the factor has not yet been identified, Kunizane et al.\(^9\) have recently reported that some flavonoids of glycosylated form were effective in mice. Flavonoids in a diet, for instance phloretin and quercetin glycoside, have been reported to be absorbed from the gut and to be present as glycosides in human plasma.\(^9\) It is therefore likely that some flavonoids contained in cabbage may stimulate the productivity of TNF and IL-1. In contrast to the effect on normal rats, the oral administration of cabbage juice to hepatoma-bearing rats failed to stimulate TNF and IL-1 production. Since AH109A implantation has been found to cause an increase in TNF and IL-1 production,\(^9\) the stimulatory action of cabbage on the production of both cytokines that was observed in the normal state might have been masked in the hepatoma-bearing state.

In conclusion, the oral administration of cabbage juice enhanced TNF and IL-1 productivity in resident peritoneal macrophages from normal rats. Further studies are needed to identify the effective component(s) in cabbage and to clarify the mode of action.

**References**