Differential Effects of Foods Traditionally Regarded as 'Heating' and 'Cooling' on Prostaglandin E₂ Production by a Macrophage Cell Line

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Key Words

COX-2 · Food · Macrophage · PGE₂ · Prostaglandin production · Traditional food belief

Abstract

Some components of natural foods may enhance or inhibit prostaglandin formation and potentially affect the inflammation condition. A macrophage cell line, RAW264.7, was employed to examine the effects of foods traditionally regarded as 'heating' or 'cooling' on the production of PGE₂, a well-known proinflammatory mediator. Foods traditionally regarded as 'heating' (litchi, longan, and dried longan) or 'cooling' (chrysanthemum flower, bitter gourd, and lotus seed plumule) were extracted sequentially with water and ethyl acetate. The water extracts (WE) and ethyl acetate extracts (EAE) were applied to RAW264.7 macrophages in the presence or absence of LPS (lipopolysaccharide). In the absence of LPS, the WEs from the 'heating foods', litchi, longan, or dried longan had a dose-dependent enhancing effect on PGE₂ production, with respective EC₅₀s of 8.4, 16, and 11 mg/ml. This effect was accompanied by significant induction of COX-2 protein expression, as shown by Western blot analysis. In contrast, LPS-induced PGE₂ production was inhibited in a dose-dependent manner

by the WEs of the 'cooling foods', chrysanthemum flower, bitter gourd, and lotus seed plumule, with respective IC₅₀s of 0.6, 0.13, and 0.08 mg/ml. At the concentrations tested, none of the EAEs had any effect on basal PGE₂ production, while LPS-induced PGE₂ production was inhibited or increased by the EAE from bitter gourd and longan, respectively. Water-soluble extracts of foods traditionally regarded as 'heating' enhanced basal PGE₂ production, while those from 'cooling' foods significantly inhibited LPS-induced PGE₂ production by the macrophage cell line. This subject merits further study to determine whether appropriate food selection may help patients suffering from chronic inflammatory conditions.

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Introduction

Prostaglandins (PGs), a family of intercellular and intracellular messengers derived from 20-carbon polyunsaturated fatty acids, are synthesized in a wide range of tissue types and serve as autocrine or paracrine mediators signaling changes in the immediate environment. In humans, PGs are involved in diverse functions, including blood clotting, ovulation, initiation of labor, bone metabolism, nerve growth and development, wound healing,

kidney function, blood vessel tone, and the immune response [42]. The key regulatory enzyme of PG biosynthesis is cyclooxygenase (COX, or prostaglandin H synthase, PGHS), a bifunctional enzyme catalyzing the oxidative cyclisation of the central 5 carbons in 20-carbon polyunsaturated fatty acids and subsequent peroxidation [27, 55]. The final PGs produced vary, depending on the downstream enzymatic machinery present in a particular cell type.

Nonsteroidal anti-inflammatory drugs (NSAIDs) had been used in humans for many decades before Vane [53] proposed that the inflammation suppression produced by NSAIDs might be due to inhibition of COX, resulting in less proinflammatory PG production at the injury site. Molecular cloning studies have identified two forms of COX [15], COX-1, a constitutive form responsible for 'housekeeping' functions, and COX-2, an inducible form that mediates many of the inflammatory and inducible effects. Both forms carry out essentially the same catalytic reaction and have similar primary sequences [15, 48]. Inhibitors acting only on the inducible COX-2 would therefore be ideal as NSAIDs free of side effects [11].

PGE₂ is the major PG produced by macrophages. COX-2 expression in macrophages is induced in response to intrinsic factors, such as cytokines, or extrinsic factors, such as lipopolysaccharide (LPS), leading to the production of PGE₂ [45, 18]. PGE₂ produced by macrophages is a well-known pro-inflammatory mediator [17].

Some components of natural foods may enhance or inhibit PG formation and potentially affect the inflammation condition. For example, phytochemicals and other components in foods may modulate COX-2 expression and PG formation [1, 5, 9, 20, 29, 32, 35, 50, 54]. It is therefore worth screening the effect of foods on COX-2 expression and PG formation which can provide useful information on food selection for the amelioration of the pathophysiological conditions of inflammation.

For 2,000 years, people in Chinese and Indian societies have believed that certain foods are either 'heating' (or 'fire increasing') or 'cooling' (or 'fire reducing') in the body when eaten [2, 7, 14]. In addition, some physiological conditions or disease states are categorized as 'hot' or 'cold'; but this is not related to body temperature. According to traditional Chinese medicine, symptoms for the diagnosis of a 'heating' disease state include a dry mouth, thirst, a bitter taste sensation even when not eating or drinking, flushing, constipation, a small amount of dark-colored urine, a rough yellow tongue, and a fast pulse [34]. Fever, overstimulation, inflammation, and sore throat have also been described as conditions with excess heat in

the body [2]. In addition, acne or pimples, hemorrhoids, and nosebleeds are also thought to be associated with an excess consumption of heating food [22]. In contrast, disease states in which the patient is thirsty but is not willing to drink, and has pallor, diarrhea, a large amount of diluted urine, a smooth white tongue and a slow pulse are diagnosed as 'cooling' [34]. Weakness, tiredness, cold body temperature and shivering are also reported to be 'cooling' conditions [2, 14]. In agreement with the Chinese belief of yin and yang, health is thought to result from a proper balance of 'heating' and 'cooling' foods and activities, and illness is treated with foods or medicines with properties opposite to those of the disease [2, 14, 33]. In Taiwan, Hong Kong and among Chinese immigrants in the USA, in which almost all of the population accept modern medical care, the concept of 'heating' and 'cooling' foods is still prevalent in all socioeconomic classes. Many individuals select food to avoid suffering from unfavorable and disturbing conditions, often discounted by most modern medical doctors [22, 33]. This is because these traditional rules filled the explanatory and behavior niches left open in Western medicine [22].

Not all foods are categorized as 'heating' or 'cooling'; a large variety of foods such as staple foods like plain rice, noodles and steamed bun are neutral [2, 7, 33]. Moreover, foods can change categories as a result of different cooking methods [2, 7, 33]. For example, roasted and fried peanuts are typical 'heating' foods, but boiled and steamed peanuts are not. Fried foods and meat cooked in black sesame oil with ginger are also good examples of 'heating' foods produced by a cooking method. Most 'cooling' foods are of plant origin, especially fruits and vegetables. Many, but not all, of the 'cooling' fruits and vegetables have a high water content, e.g. watermelon and radish, and excretion of a large volume of diluted urine is considered one of the 'cooling' conditions. However, not all vegetables are 'cooling'; many are neutral, and a few fruits, such as litchi, longan, and durian, are considered to be 'heating'.

In spite that food items listed as 'cooling' or 'heating' may vary among societies or populations in different geographical areas because of different climates, environments and agricultural products available, the central rule of the hot/cold system prevails. In addition, susceptibility also varies among individuals which is partially attributed to an innate 'hot' or 'cold' physical nature. Although there has been speculation on the significance and rationale of the 'hot/cold' food belief from a social science aspect [2–4, 14], an empirical rational basis has not been reported. A chemical feature common to 'hot' or 'cold' foods cannot

be ruled out simply based on the nutrient composition, implying that some components with special functions may be involved. On the other hand, the class of body regulator that is targeted by 'hot' or 'cold' foods and mediates the diverse 'hot/cold' syndrome must be one that has a wide range of functions or can trigger diverse physiological changes in the body.

Since (1) the extreme case of a 'heating' condition resembles inflammation in certain ways, and (2) feeding mice a diet containing frying oil tended to increase PGE₂ production by peritoneal macrophages [26] and fried foods are very common 'heating' foods [2, 33], the effect of some typical 'heating' and 'cooling' foods on PGE₂ production was tested using a murine macrophage cell line, RAW 264.7, as an in vitro model [38]. The 'heating' foods, litchi, longan, and dried longan [2], and the 'cooling' foods, bitter gourd [14], chrysanthemum flower [2], and lotus seed plumule, were chosen for testing because these are natural foods in which the classification of 'heating' or 'cooling' is most widely accepted. Results of this study will provide basic information supporting further well-controlled in vivo experiments.

Materials and Methods

Materials

The 'heating' foods chosen for this study were longan (*Nephelium longana* Camb or *Euphoria longana* Lam), litchi (or lychee, leechie or lichi, *Litchi sinensis* Sonn), and dried longan (during drying, longan undergoes a marked browning reaction), while the 'cooling' foods chosen were dried chrysanthemum flower (*Chrysanthemum morifolium* Hemsl.), fresh bitter gourd (*Momordica charantia* L.), and dried plumule of lotus seed (*Nelumbo nucifera* Gaertn). Longan, litchi, and bitter gourd, which are common fruit and vegetables in Taiwan, were purchased from a local market. Dried chrysanthemum flower and lotus seed plumule were purchased from a Chinese traditional medicine store. Dried longan, the hot-air-dried product of the whole longan fruit including the peel, was purchased from a grocery store.

The RAW264.7 macrophage cell line (CCRC60001, originally from the American Type Culture Collection; designation, TIB-71) was obtained from the cell bank of the Food Industry Research and Development Institute, Hsin Chu, Taiwan. Dulbecco's minimal essential medium (DMEM) and fetal bovine serum were purchased from Gibco (Md., USA).

Methods

Extraction of Food Samples. The edible portions of longan, litchi, dried longan (the aril), and bitter gourd (the flesh) were cut into small pieces and homogenized in a Waring blender with a minimal amount of double-distilled water, and the homogenates were filtered through several layers of gauze. Dried chrysanthemum flower was boiled in water (10 ml/g flower) for 5 min and filtered. Then the residue was reextracted in the same way and the two filtrates were combined. The dried lotus seed plumule was homogenized with water (10 ml/g plu-

mule) and filtered. All filtrates were centrifuged at 10,500 g at 4°C for 30 min and the clear supernatant, the water extract (WE), was freeze-dried. The ethyl acetate extract (EAE) was prepared by freeze-drying the precipitate and filtered residues from the water extraction and extracting it overnight by shaking at room temperature with ethyl acetate (30 ml/g of residue), followed by filtration and drying of the filtrate in a rotary evaporator (Büchi, Essen, Germany). The yields of dried water extract of longan, litchi, dried longan, bitter gourd, lotus seed plumule and chrysanthemum were 7.15, 14.02, 43.49, 2.76, 28.95 and 34.5 g/100 g of the fresh edible portion (longan, litchi and bitter gourd) or dried material (dried longan, lotus seed plumule and chrysanthemum). The yields of evaporated EAE of longan, litchi, dried longan, bitter gourd and lotus seed plumule were 1.53, 4.01, 2.23, 1.86, and 17.25 g/100 g of the dried residue after water extraction.

For cell culture experiments, the WEs were dissolved in DMEM, while the EAEs were dissolved in a minimal amount of ethanol or dimethyl sulfoxide. Both were diluted to the appropriate concentration with DMEM immediately before use.

Cell Culture

RAW 264.7 cells were grown in 25-cm² flasks in DMEM containing 10% fetal bovine serum at 37°C in a 5% CO₂ atmosphere. Near-confluent cells were removed with a cell scraper, seeded on 96-well plates at a concentration of 6×10^5 cell/ml, incubated for 24 h at 37°C in 5% CO₂, and then washed with phosphate-buffered saline (27 mM, pH 7.4, PBS). To test the effect of food extracts on PGE₂ production, the cells were incubated for 18 h at 37°C with serum-free DMEM containing various concentrations of sample in the presence or absence of 1 ng/ml of LPS. Then the medium was collected and stored at -70°C until analysis for PGE₂. Cell viability was assayed using the MTT method [39]. Experiments were repeated at least three times in triplicate.

To study the effect of the above-mentioned treatment on COX-2 protein expression, confluent cells were seeded on 6-cm² dishes and treated for 12 h at 37°C, in the presence or absence of 1 ng/ml of LPS, with WEs or EAEs at the concentration that showed a maximal effect on PGE₂ production and did not significantly affect cell viability. The cells were then washed twice with PBS, suspended in 1 ml of PBS using a cell scraper, and transferred to a microcentrifuge tube. After centrifugation, the supernatants were discarded and the cell pellets stored at -70°C until analyzed by Western blotting.

Analyses

The collected medium was diluted to an appropriate concentration and analyzed for PGE₂ by competitive enzyme-immunoassay using a commercial kit (Cayman, Ann Arbor, Mich., USA). For COX-2 protein analysis, cell pellets were lysed and the cellular proteins extracted, separated by polyacrylamide gel electrophoresis, and transferred to a polyvinylidene fluoride membrane, which was then treated sequentially with primary antibody (rabbit anti-COX-2 antiserum, Cayman), secondary antibody (alkaline-phosphatase-conjugated anti-rabbit antibody, Tropix, Applied Biosystems, Foster City, Calif., USA), and CDP-star (chemiluminescent substrate of alkaline phosphatase, Tropix). The specific protein band was visualized using X-ray film and quantitated by image analysis.

Statistical Analysis

The data for PGE₂ production were expressed as the mean $\pm$ SD for triplicate wells in a representative experiment. The significance of

differences at each sample concentration was analyzed by ANOVA and Duncan's multiple range test using SAS 5.1 software. To calculate the percentage enhancement of basal PGE₂ production by a sample at a specific concentration, the maximal PGE₂ production produced by this sample was taken as 100% and the enhancing effect calculated using the equation: percent enhancement = $[(PGE_2 \text{ sample} - PGE_2 \text{ basal}) / (PGE_2 \text{ maximum} - PGE_2 \text{ basal})] \times 100\%$, where PGE₂ basal is the PGE₂ produced by nonstimulated untreated macrophages and PGE₂ sample is the PGE₂ produced in the presence of a specific concentration of the sample. The EC₅₀ value, calculated from the curve of

percentage enhancement versus concentration, is the concentration of sample that produces 50% enhancement. To calculate the percentage inhibition of LPS-stimulated PGE₂ production, PGE₂ production in the presence of 1 ng/ml of LPS was taken as 0% inhibition and basal PGE₂ production as 100% inhibition; the percentage inhibition by a sample at a specific concentration was calculated as: percentage inhibition = $[1 - (PGE_2 \text{ sample} + LPS - PGE_2 \text{ basal}) / (PGE_2 \text{ LPS} - PGE_2 \text{ basal})] \times 100\%$. The IC₅₀ value, calculated from the curve of percentage inhibition versus concentration, is the concentration of sample that results in 50% inhibition.

Fig. 1. Effect of WEs on basal PGE₂ production. **A** Chrysanthemum flower. **B** Bitter melon. **C** Lotus seed plumule. **D** Longan. **E** Dried longan. **F** Litchi. RAW264.7 cells (6×10^4 cells/well) were seeded on 96-well plates and grown for 24 h, then incubated for 18 h with DMEM or DMEM containing various concentrations of WE. PGE₂ in the medium was then measured by competitive enzyme-immuno-

assay. The data are the mean $\pm$ SD for triplicate wells from a representative experiment. The experiment was repeated at least three times with similar results. F0 indicates basal PGE₂ production when cells were incubated in DMEM alone. Means not sharing a common letter (a–d) were significantly different ($p < 0.05$) when analyzed by ANOVA and Duncan's multiple range test.

Fig. 2. Effect of WEs on LPS-induced PGE₂ production. **A** Chrysanthemum flower. **B** Bitter gourd. **C** Lotus seed plumule. **D** Longan. **E** Dried longan. **F** Litchi. RAW264.7 cells (6×10^4 cells/well) were seeded on 96-well plates and grown for 24 h, then incubated for 18 h with DMEM containing 1 ng/ml of LPS with or without various concentrations of the WEs. The PGE₂ concentration of the medium was

measured using an enzyme-immunoassay kit. The data shown are the mean $\pm$ SD for triplicate wells of a representative experiment. The experiment was repeated at least three times with similar results. Means not sharing a common letter (a–f) were significantly different ($p < 0.05$) when analyzed by ANOVA and Duncan's multiple range test.

Results

The basal level of PGE₂ production by nonactivated macrophages (without LPS or any other stimulation) was low and in the range of 0.5–1 ng/ml. As expected, treat-

ment with 1 ng/ml of LPS significantly increased the expression of COX-2 protein and PGE₂ production.

In this study, a wide range of concentrations of sample extracts was tested, but the data shown are restricted to those for experiments in which the concentration of

extract did not significantly affect cell viability, as determined by the MTT assay. The enhancing and inhibitory effects described below were therefore not secondary to any change in cell number or viability.

Effects of Food Extracts on PGE₂ Production by RAW264.7 Cells

Water Extracts. The effects of the different WEs on basal PGE₂ production were first tested. As shown in figure 1, basal PGE₂ production was unaffected by WEs from chrysanthemum flower (fig. 1A), bitter gourd (fig. 1B), and lotus seed plumule (fig. 1C), but was increased in a dose-dependent manner by WEs from longan (fig. 1D), dried longan (fig. 1E), and litchi (fig. 1F). At a concentration of 50 mg/ml, longan WE increased PGE₂ production 8.5-fold compared with the basal level, while, at 20 mg/ml, the WEs of dried longan and litchi resulted in 5- and 7.5-fold increases, respectively, over the basal levels. The EC₅₀s for the WE of longan, dried longan, and litchi were 16, 11, and 8.4 mg/ml, respectively.

The WEs were then tested for their effect on LPS-induced PGE₂ production. As shown in figure 2, WE from chrysanthemum flower (fig. 2A), bitter gourd (fig. 2B), and lotus seed plumule (fig. 2C) inhibited LPS-induced PGE₂ production in a dose-dependent manner. At a concentration of 2 mg/ml, the WE from chrysanthemum flower completely inhibited PGE₂ production, while the WEs from bitter gourd (0.5 mg/ml) and lotus seed plumule (0.2 mg/ml) produced 68 and 63% inhibition, respectively. At a concentration equal to or greater than 1.0 (bitter gourd) or 0.5 mg/ml (lotus seed plumule), the WEs significantly reduced cell viability. The IC₅₀s for the WEs from chrysanthemum flower, bitter gourd, and lotus seed plumule were 0.6, 0.13, and 0.08 mg/ml, respectively. In contrast, the WEs from longan (fig. 2D), dried longan (fig. 2E), and litchi (fig. 2F) did not inhibit LPS-induced PGE₂ production, but instead tended to increase it.

Ethyl Acetate Extracts. Due to their limited solubility, the EAEs could only be tested in the µg/ml concentration range. At these levels, none of the EAEs significantly altered basal PGE₂ production (data not shown). However, when tested for effects on LPS-induced PGE₂ production, the EAE from bitter gourd showed dose-dependent inhibition (IC₅₀ = 21 µg/ml, fig. 3A), and, at concentrations of 50–100 µg/ml, caused almost complete inhibition, while the EAE from longan resulted in increased PGE₂ production (fig. 3C).

Effects of Food Extracts on COX-2 Protein Expression by RAW264.7 Cells

Levels of COX-2 protein in RAW 264.7 cells treated with various samples in the presence or absence of LPS were determined by Western blot analysis. As shown in figure 4A, WEs from litchi, longan, dried longan, and lotus seed plumule induced significant COX-2 protein expression in nonstimulated macrophages (without LPS). None of the WEs had any significant effect on the amount of COX-2 protein induced by 1 ng/ml of LPS (data not shown); however the EAEs from lotus seed plumule and longan (100 µg/ml) showed a significant reduction (fig. 4B). As no significant effect on PGE₂ production was seen when RAW264.7 cells were treated with EAE alone, the effect of EAEs on basal COX-2 protein was not checked.

Discussion

Among the six foods tested in this study, WEs from the 'heating' foods, longan, litchi, and dried longan, significantly enhanced basal PGE₂ production by the macrophage cell line RAW264.7 and also tended to increase LPS-induced PGE₂ production. In contrast, WEs from the 'cooling' foods, bitter gourd, chrysanthemum flower, and lotus seed plumule, did not stimulate basal PGE₂ production and inhibited LPS-induced PGE₂ production. These results support our hypothesis that 'heating' or 'cooling' foods may, respectively, stimulate or inhibit PGE₂ production by macrophages. It is therefore worth testing these effects further in an appropriate in vivo model and also testing more foods for these effects.

The yields of dried water extract of longan, litchi and bitter gourd corresponded to a WE concentration of 71.5, 140 and 27.6 mg/g of the fresh edible portion. This is higher than their respective EC₅₀s of 8.4 (litchi) and 16 mg/ml (longan), and IC₅₀ of 0.13 mg/ml (bitter gourd). Similarly, the EC₅₀ of WE from dried longan (11 mg/ml) and IC₅₀s of the chrysanthemum flower (0.6 mg/ml) and lotus seed plumule (0.08 mg/ml) were lower than the WE concentration in 1% (wt/vol) chrysanthemum or dried lotus seed plumule beverages or a 3% (wt/vol) dried longan beverage. After eating, the presence of the active component at a sufficient concentration, at least in the gastrointestinal tract, is therefore possible.

Since the water-soluble component(s) of longan, litchi, and dried longan stimulated PGE₂ production by the macrophage cell line and also tended to increase the expression of COX-2 protein (fig. 4 A), it is probable that

Fig. 3. Effect of EAEs on LPS-induced PGE₂ production. **A** Bitter gourd. **B** Lotus seed plumule. **C** Longan. **D** Dried longan. **E** Litchi. The EAEs were dissolved in a minimal amount of dimethylsulfoxide or ethanol, then diluted with DMEM. The experiment was performed as in figure 2. Means not sharing a common letter (a–d) were significantly different ($p < 0.05$) when analyzed by ANOVA and Duncan's multiple range test.

the enhancing effect on PGE₂ production involved induction of COX-2 expression.

COX-2 expression is induced in macrophages in response to intrinsic factors such as cytokines, or extrinsic factors such as LPS, leading to the production of PGE₂ [18, 45]. Most of these factors are molecules that can trig-

ger the signaling pathway by binding to receptor molecules in the cell membrane. For example, LPS acts on macrophages by binding to LPS binding protein and mCD14, a receptor located on the macrophage surface membrane [51]. This activates the signal transduction pathway, which includes protein kinase C [12] and

Fig. 4. Western blot analysis of COX-2 protein expression after treatment with extracts. **A** Treatment with WE or LPS alone: lane 1 = F0 (basal level, no treatment); lane 2 = F0L1, LPS alone (1 ng/ml); lane 3 = bitter gourd WE alone (0.2 mg/ml); lane 4 = dried longan WE alone (5 mg/ml); lane 5 = litchi WE alone (20 mg/ml); lane 6 = lotus seed plumule WE alone (0.2 mg/ml); lane 7 = longan WE alone (20 mg/ml). **B** Treatment with LPS (1 ng/ml) with or without EAE: lane 1 = F0 (basal level, no treatment); lane 2 = F0L1 (LPS alone); lane 3 = LPS plus dried longan EAE (10 µg/ml); lane 4 = LPS plus litchi EAE (20 µg/ml); lane 5 = LPS plus bitter gourd EAE (100 µg/ml); lane 6 = LPS plus lotus seed plumule EAE (200 µg/ml); lane 7 = LPS plus longan EAE (20 µg/ml); lane 8 = LPS plus longan EAE (100 µg/ml).

RAW 264.7 cells (4×10^6 /ml) were seeded on a 6-cm² dish and grown for 24 h. Then the medium was changed to DMEM or LPS alone or DMEM containing EAEs and LPS. The cells were then incubated for 12 h and harvested for Western blot analysis of COX-2 protein expression. The upper panels show Western blots from a representative experiment; the experiment was repeated three times with similar results. The lower panels show image analysis results; the data are the means $\pm$ SD for three experiments. * $p < 0.05$ vs. F0 (cells with no treatment) and + $p < 0.05$ vs. F0L1 (cell treated with LPS only). In the lower panel of **A**, the p value of the difference between longan (lane 7) and F0 (Lane 1) was 0.059, i.e. nearly significant.

MAPK [18]. Expression of the COX-2 gene is induced when these signals activate transcriptional factors, such as NF- κ B [10, 18, 31]. Theoretically, molecules able to bind to cell surface receptors, trigger the signaling pathway, or activate transcription factors could directly or indirectly induce COX-2 protein expression and result in PGE₂ production. Molecules that interfere with any point in the above-mentioned mechanism of action of LPS may lead to inhibition of PGE₂ production [19]. Other potential factors that could influence PGE₂ production include those affecting phospholipase A₂ activity [57] or the fatty acid composition of the cell membrane [32], both of which may alter substrate availability, or those that may directly affect the catalytic activity of COX-2 [6].

Among the dietary and food factors that may affect PGE₂ production, direct inhibition of COX activity has been demonstrated for resveratrol [20, 35, 50], hydroxy-

stilbenes [47], and curcumin [1]. Enhancement of expression of COX-2 protein has also been demonstrated by n-6 fatty acids in the mammary gland [5]. Inhibition of COX-2 expression in macrophages, epithelial cells, the RAW 264.7 macrophage cell line, and colorectal cancer cells has been reported to be caused, respectively, by genistein [10], resveratrol [50], apigenin [29], and antioxidants [9]; the first three agents are bioflavonoids with antioxidant activity [44] and it appears that the antioxidant activity is closely related to the inhibitory effect on COX [35, 37, 52]. Using the same testing system as that used in this study, we have observed a significant inhibition on the LPS-induced PGE₂ production by a number of phytochemicals including curcumin, quercetin, apigenin, narigenin, resveratrol, and some tea polyphenols [54].

For chrysanthemum flower, we only tested the WE, since it is usually ingested as a drink prepared by brewing in hot water. When cells were treated with chrysanthemum flower WE, although inhibition of PGE₂ production was seen in the presence (fig. 2A) of LPS, no significant effect was seen on COX-2 protein expression (data not shown). These results suggest that chrysanthemum flower WE may directly interfere with COX-2 enzyme activity. Compounds isolated from chrysanthemum flowers, including sesquiterpenes and flavones, have been shown to inhibit LPS-induced nitric oxide production by macrophages [56] and to be antioxidative and inhibit cancer cell growth [41]. Some flavones may be partially soluble in water. It is not known if antioxidant flavonoids are responsible for this inhibition by chrysanthemum flower WE.

Numerous biologically active components have been identified in bitter melon, also known as bitter melon. For example, seed extracts contain MAP-30, an anti-HIV and anti-tumor protein [28], momorcharins [43] and momordins [21], which inactivate ribosomes, and momordin Ic [30] and oleanolic acid glycoside [36], both of which can alter gastrointestinal transit time and blood glucose. An extract of bitter melon fruit has been shown to have hypoglycemic activity [46] and to alter the activities of phase I and phase II drug metabolizing enzymes in the rat liver [25]. In this study, the extract was prepared exclusively from the fleshy part with the seeds removed. Both the WE and EAE from bitter melon caused dose-dependent inhibition of LPS-induced PGE₂ production; again, since COX-2 protein expression did not change significantly, inhibition of COX-2 enzyme activity may have been responsible.

Lotus seed plumule WE showed a marked inhibitory effect on PGE₂ production on a dried weight basis. However, its effect on PGE₂ production and COX-2 protein expression was complex, since it significantly inhibited LPS-induced PGE₂ production (fig. 2C), but did not affect COX-2 protein expression (data not shown). On the other hand, a faint COX-2 protein band was seen on the Western blot of RAW264.7 cells treated with lotus seed plumule WE in the absence of LPS (fig. 4 A) and this was accompanied by a very slight increase in PGE₂ production (fig. 1C). The most intriguing result was the effect of the EAE, which significantly reduced COX-2 protein expression (fig. 4B), but did not significantly affect PGE₂ production (fig. 3 B) by RAW264.7 cells treated with LPS. These results suggest that multiple compounds in lotus seed plumule may exert diverse, or even opposite, effects on the RAW264.7 macrophage cell line. Despite this com-

plicated result, the inhibitory effect of lotus seed plumule WE on LPS-induced PGE₂ production is considered most significant, since brewing in hot water to prepare a drink is the most common method used for the preparation of this food material.

Depending on the nature of the inhibitor and the system used, COX inhibitors have been shown to reduce [12, 24, 29, 50], induce [40], increase [8], or have no effect on [6] levels of COX-2 mRNA or protein. Dietary bioflavonoids, such as genistein [12], apigenin [29], and resveratrol [50], inhibit COX-2 gene expression, probably by interfering with the signal transduction pathway that leads to expression of COX-2 genes. In contrast, COX inhibitors, such as indomethacin or NS398, can induce COX-2 mRNA expression [40] in a manner similar to peroxisome proliferators, or increase COX-2 protein levels [8], probably by stabilization of the enzyme protein. PGE₂ can destabilize the COX-2 protein [40]. In the presence of LPS, the inhibition of PGE₂ production without a change in COX-2 protein levels produced by extracts of bitter melon, chrysanthemum flower, and lotus seed plumule are similar to the results seen with NSAID reported by Barrios-Rodiles et al. [6].

The molecule(s) in litchi, longan, and dried longan responsible for induction of COX-2 expression in the macrophage remain to be elucidated. Because of their water-soluble nature, plant lectins may be candidates. Lectins from various plant sources have been shown to bind to macrophage surface glycoproteins [16, 23] and can result in activation of the macrophages [23]. Further studies are needed to test this possibility.

Macrophages represent a system of widely dispersed cells that are able to recognize and destroy invading microorganisms and altered host components, such as apoptotic cells. The macrophage plays an important role in the regulation of the immune response [13]. Fixed macrophages, such as alveolar macrophages, Kupffer cells, histocytes, mesangial cells, and microglial cells, like free macrophages, are monocyte-derived cells and may serve additional functions in the tissues in which they reside, respectively. For example, mesangial cells are involved in the regulation of the glomerular filtration rate and renal blood flow [49]. In addition, the final products of COX, such as PGE₂ and other prostaglandins, are involved in diverse physiological functions and pathophysiological conditions. The results of this study provide one of several possible rationales for the effects of 'heating' and 'cooling' foods. However, given the diverse aspects of physiological conditions associated with 'heating' and 'cooling', other possible mechanisms of action await further discovery.

For example, diuretic and antidiuretic actions may be physiological effects of these so-called 'heating' and 'cooling' foods.

In conclusion, WEs of foods traditionally regarded as 'heating', namely litchi, longan, and dried longan, increased COX-2 protein levels, and basal PGE₂ production by the RAW264.7 macrophage cell line. In contrast, WEs of foods traditionally regarded as 'cooling', namely chrysanthemum flower, bitter melon, and lotus seed plumule, significantly decreased LPS-induced PGE₂ production by these cells. These results provide preliminary evidence for a scientific basis for the ancient oriental belief in 'heating' and 'cooling' foods. As PGE₂ produced by macrophages is

an important pro-inflammatory agent, this subject merits further study, as this could provide useful information on food selection as a means of treatment for the pathophysiological condition of inflammation.

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