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Pharmacological evaluation of tea polysaccharides with antioxidant activity in gastric cancer mice

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ABSTRACT

Tea polysaccharides were fractionated by Sephadex G-100 gel permeation chromatography. The results showed that tea polysaccharides were mainly composed of TF-1, TF-2 and TF-3. The average molecular weights of TF-1, TF-2 and TF-3 determined by high-performance gel permeation chromatography (HPGPC) and high performance liquid chromatography-evaporative light scattering detector (HPLC-ELSD) were 231,568 Da, 46,278 Da and 7251 Da, respectively. The monosaccharide composition of Renshen polysaccharides was evaluated by GC. TF-1 was composed of glucose, mannose, xylose with molar ratio of 1:3.2:1.4. TF-2 and TF-3 consisted of glucose, xylose and glucose, xylose, arabinose with molar ratios of 1:1.7 and 1:2.5:0.9, respectively. TF-1 contained mannose as main sugar component and TF-2 was rich in xylose, whereas TF-3 was rich in xylose. In addition, tea polysaccharides could decrease stomach malondialdehyde (MDA) level, serum interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α) levels, increased serum immunoglobulin A (IgA), immunoglobulin G (IgG), immunoglobulin M (IgM), interleukin-2 (IL-2), interleukin-4 (IL-4), interleukin-10 (IL-10) levels, and stomach antioxidant enzymes activities in gastric cancer mice.

1. Introduction

Tea, a product made up from leaf and bud of the plant *Camellia sinensis*, is the most widely consumed beverage in the world and has become an important agricultural product. Originating from China, tea was first cited as early as in 100 B.C. in the Shen Nong's Herbal Classic (widely considered as the oldest book on oriental herbal medicine and the foundation of traditional Chinese medicine) for its detoxification effects (Cai, 1979; Yan, Wang, & Wang, 1998). Lower grade green tea has traditionally been used for the treatment of diabetes in East Asia, especially in Japan (Cai, 1979; Takeo & Kinugasa, 1992). Earlier studies have shown that green tea and its polyphenol constituents are effective antioxidants and have pharmacological activities, such as anti-cancer, antimutation and anti-atherosclerosis (Andrea & Michael, 1997; Chi, 1997; Zhu, Wang, & Guo, 2001). It has been reported that the polysaccharide conjugates isolated from green tea have significant biological activities such as immunological, antiradiation, antiblood coagulation, anticancer, anti-HIV, and hypoglycemic activities (Isiguki, Takakuwa, & Takeo, 1991; Mori & Morita, 1988; Shimizu et al., 1988; Takeo, Unno, Kinugasa, Yayabe, & Motoyama, 1998; Xie & Nie, 2006).

Gastric cancer is one of the most common malignant diseases and the second leading cause of cancer related death worldwide (Hu et al., 2004; Pisani, Parkin, & Bray, 1999). Notwithstanding the global declining incidence of gastric cancer, mortality is still rising in Asian countries (*e.g.*, China, Japan, and South Korea) (Hu et al., 2004). In recent years, the development of new anti-cancer drugs has been a key issue in cancer chemotherapy, as cancer cells that are resistant to current chemotherapy will eventually dominate the cell population, resulting in much higher mortality (Kang et al., 2005). Epidemiological studies have provided strong evidence to suggest that a diet containing fruits and vegetables may contribute to cancer risk reductions in humans (Jayaprakasha et al., 2008). Several plant products contained in foods exert anti-cancer effects in humans. Juice or freeze-dried powder from Brussels sprouts significantly enhances apoptosis levels and reduces mitosis in the colonic crypts of an animal model (Smith, Mithen, & Johnson, 2003). Moreover, many flavonoids found in fruits and vegetables, quercetin for example, induce apoptosis in cancer cells (Chen, Wu, & Lin, 2007).

The aim of this study was to investigate the antioxidant potential of tea polysaccharides using gastric cancer mice model.

2. Materials and method

2.1. Purification

Proteins and nucleic acid has been removed in tea polysaccharides. The crude polysaccharide was subjected to gel filtration on a column (1.5 cm × 100 cm) of Sephadex G-100, eluted with distilled water and monitored using the phenol-sulfuric acid

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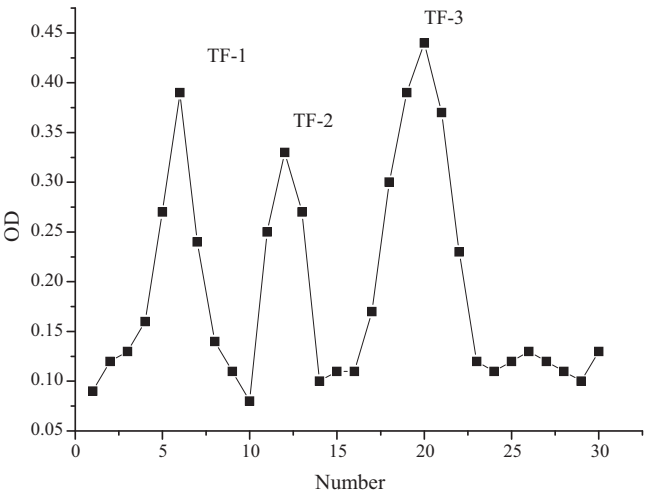

Fig. 1. TF-1, TF-2 and TF-3 fractions.

method (Xi, Wei, Wang, Chu, & Xiao, 2010). The flow rate was 0.2 ml/min.

2.2. Molecular weight determination

The homogeneity and molecular weight of fractions were identified by high-performance gel permeation chromatography (HPGPC) with a Waters HPLC apparatus (UK6 injector and 510 HPLC pump, Waters, Milford, MA) equipped with an UltrahydrogelTM-500 column (300 cm × 7.8 mm), a Waters 2410 RI detector, and UV detector connected in series with a Millennium32 workstation. Detailed experimental conditions were as follows: concentration of sample, 1 mg/ml, column and RI detector temperature, 35 °C (column temperature auto-control system); injection volume, 20 μl; mobile phase, ultra-pure water; flow rate, 0.6 ml/min; run time, 30 min, and integral pattern, force baseline to peak. Different weight-average molecular weights of standard dextrans, T-2000, T-500, T-70, T-40, and T-10, were prepared as 0.1% (w/v) solutions and 20 μl of solutions were injected in each run, and then the retention time was plotted against the logarithms of their respective molecular weights. A calibration curve was prepared from the known MW Dextran T system standards.

2.3. Component analysis

The monosaccharide composition of fractions was determined. Briefly, sample (10 mg) was hydrolysed with 1 ml of 2 M trifluoroacetic acid (TFA) at 120 °C for 2 h in a sealed tube. After that, the removal of the excess amount of TFA was accomplished by co-evaporation at reduced pressure with ethyl alcohol added after reaction. The subsequent treatment of the resultant dry hydrolysate with acetic anhydride and pyridine afforded the corresponding alditol acetate which was analyzed by an Agilent 6890 N gas chromatography (Agilent, Santa Clara, CA, USA) fitted with a flame ionization detector (FID) and an AJW&HP-88 capillary column (100 mm × 250 μm, 0.25 μm). The analytical condition was 3 min at 180 °C, from 180 °C to 230 °C at 10 °C/min and held for 20 min at 230 °C, from 230 °C to 240 °C at 5 °C/min and held for 20 min at 240 °C, and from 240 °C to 250 °C at 5 °C/min and held for 5 min at 250 °C. Quantification was carried out from the peak area.

2.4. Animals and treatment

Kunming mice, weighing 26 ± 2 g were housed under standard environmental conditions (23 ± 1 °C, 55 ± 5% humidity and a 12 h light/dark cycle) and maintained with free access to water and a standard diet *ad libitum*. The general guidelines for the care and use of laboratory animals recommended by the Council of China were followed.

The mice were divided into 5 groups of 10 animals each. Group I served as the control and was given saline orally for 50 days. Group II were induced with MNNG 100 mg/kg body weight by oral gavage at days 0 and 14 and saturated NaCl (1 ml per mice) was given 3 days for 4 weeks and maintained till the end of the experimental period. Groups III–V were induced with MNNG + NaCl (as in group II) and treated with TP (400, 600 and 800 mg/kg body weight, dissolved in saline) simultaneously for 50 days from the first dose of MNNG + NaCl. The experiment was terminated in the 51th day, and all rats were killed after an overnight fast. Blood was collected, and the plasma separated was used for analysis. Stomachs were excised to prepare a 10% homogenate for biochemical measurements.

Serum immunoglobulin A (IgA), immunoglobulin G (IgG) and immunoglobulin M (IgM) levels were measured according to the literature (Lin, Lu, Liou, & Liou, 2006).

Serum interleukin-2 (IL-2), interleukin-4 (IL-4), interleukin-10 (IL-10), interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α) level were measured with ELISA immunity kits.

Malondialdehyde (MDA), a product of LP, was determined according to the method of Ohkawa, Ohishi, and Yagi (1979). In brief, the sample was added to 8.1% SDS, vortexed and incubated for 10 min at room temperature. This was followed by the addition of 375 μl of 20% acetic acid and 0.6% thiobarbituric acid, and placed in boiling water bath for 60 min. The samples were allowed to cool and 1.25 ml butanol–pyridine (15:1) was added and centrifuged at 640 μg for 5 min. Absorbance of the colored layer was measured at 532 nm with 1,1,3,3-tetramethoxy propane as standard. MDA concentration was expressed as nmol/mg protein.

Reduced glutathione (GSH) forms a characteristic compound with 5,5′ dithiobis 2-nitro benzoic acid (DTNB). The extent of the reaction is followed spectrophotometrically at 412 nm. GSH estimation was achieved based on the method of Sedlak and Lindsay (1968).

The activity of superoxide dismutase (SOD) was assayed by monitoring its ability to inhibit the photochemical reduction of nitroblue tetrazolium (NBT). Each 1.5 ml reaction mixture contained 100 mM Tris/HCl (pH 7.8), 75 mM NBT, 2 μM riboflavin, 6 mM EDTA, and 200 μl of supernatant. Monitoring the increase in absorbance at 560 nm followed the production of blue formazan. One unit of SOD is defined as the quantity required to inhibit the rate of NBT reduction by 50% as described by Winterbourn, Hawkins, Brian, and Carrell (1975).Glutathione peroxidase (GSH-Px) was analyzed by the method of Flohe and Gunzler (1984). 50 μl of 0.1 M phosphate buffer (pH 7.0), 100 μl enzyme sample, 100 μl glutathione reductase (0.24 units) and 100 μl of 10 mM GSH were mixed. The mixture was pre-incubated for 10 min at 37 °C followed by the addition of 100 μl of 1.5 mM NADPH in 0.1% $NaHCO_3$. The overall reaction was started by adding 100 μl of prewarmed hydrogen peroxide and the decrease in absorption at 340 nm monitored for 3 min.

2.5. Statistical analysis

Results are expressed as mean ± S.E.M. One-way analysis of variance (ANOVA) followed by Dunnett's test was used for statistical evaluation. *P* values less than 0.05 were considered significant.

3. Result and discussion

Tea polysaccharides were isolated from tea through hot water extraction and continuation 0.5 M NaOH extraction, ethanol

Table 1
Chemical composition of three fractions of tea polysaccharides.

Fractions	Molecular weight	Monosaccharides	Molar ratio
TF-1	231,568 Da	Glucose:mannose:xylose	1:3.2:1.4
TF-2	46,278 Da	Glucose:xylose	1:1.7
TF-3	7251 Da	Glucose:xylose:arabinose	1:2.5:0.9

precipitation, deproteinized by Sevag method, dialyzed against water and dried. As a result, the extraction yield of tea polysaccharides may reach to approximately 25.61% of the dried tea. Tea polysaccharides were fractionated by Sephadex G-100 gel permeation chromatography. The results showed that tea polysaccharides were mainly composed of TF-1, TF-2 and TF-3 (Fig. 1). The average molecular weights of TF-1, TF-2 and TF-3 determined by HPGPC were 231,568 Da, 46,278 Da and 7251 Da, respectively. The monosaccharide composition of Renshen polysaccharides was evaluated by GC-MS. According to Table 1, TF-1 was composed of glucose, mannose, xylose with molar ratio of 1:3.2:1.4. TF-2 and TF-3 consisted of glucose, xylose and glucose, xylose, arabinose with molar ratios of 1:1.7 and 1:2.5:0.9, respectively. TF-1 contained mannose as main sugar component and TF-2 was rich in xylose, whereas TF-3 was rich in xylose. (Table 1).

The well-known experimental carcinogen N-methyl-N′-nitro-N-nitrosoguanidine (MNNG) induces gastric cancer in male albino wistar rats which show similarities to human gastric tumors

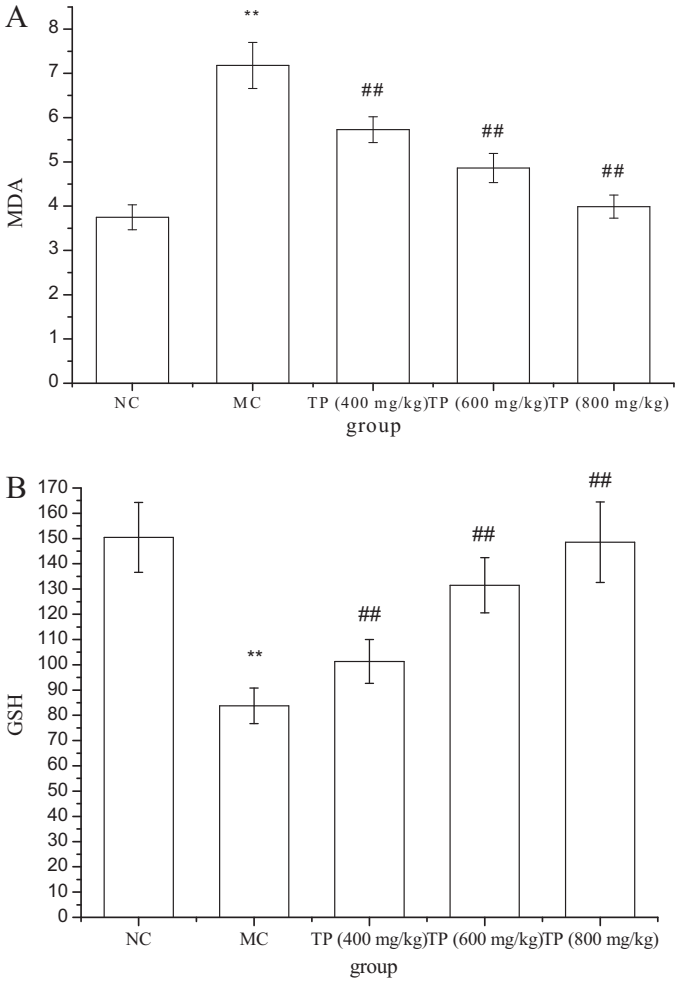

Fig. 2. Effect of tea polysaccharides on stomach MDA and GSH levels. $^{**}P < 0.01$, compared with NC group; $^{**}P < 0.01$, compared with NC group; $^{\#\#}P < 0.01$, compared with MC group.

Table 2
Effect of tea polysaccharides on SOD, CAT and GSH-Px activities.

Group	SOD	CAT	GSH-Px
NC	222.8 ± 18.7	48.25 ± 3.88	59.66 ± 4.39
MC	139.7 ± 11.9**	21.53 ± 2.07**	33.38 ± 3.08**
TP (400 mg/kg b.w.)	180.3 ± 16.5±±	32.81 ± 2.81±±	41.23 ± 3.75±
TP (600 mg/kg b.w.)	199.4 ± 20.1±±	39.79 ± 3.22±±	48.74 ± 3.93±±
TP (800 mg/kg b.w.)	220.2 ± 18.4±±	45.53 ± 4.07±±	57.32 ± 4.88±±

** $P < 0.01$, compared with NC group.
± $P < 0.05$.
±± $P < 0.01$, compared with MC group.

(Mirrish, 1975). Chemoprevention, defined as the use of nontoxic substances to inhibit or reverse the process of carcinogenesis, is now considered an essential approach to cancer prevention and/or treatment. Because gastric cancer is known to have epigenetic origins, such as infection with Helicobacter pylori and/or exposure to carcinogenic nitrosamines (Graham, 2000; Palli, 2000), it is thought to be preventable through appropriate intervention. However, at present, there are limited experimental data regarding specific agents that prevent or retard gastric carcinogenesis.

Oxidative stress associated with increased levels of LPO and other thiobarbituric acid reactive intermediates are linked to cancer progression (Kanno et al., 2005; Kumaraguruparan, Subapriya, Viswanathan, & Nagini, 2002). Superoxide dismutase is believed to protect cells, notably erythrocytes against superoxide- and hydrogen peroxide-mediated damage (Beutlar & Gelbart, 1985). Catalase is widely distributed in all tissues and catalyzes the breakdown of H_2O_2. The source of H_2O_2 is mainly SOD-mediated dismutation of SOD radicals generated by enzymatic and nonenzymatic systems. Several reports have cited decreased activities of SOD and catalase in various carcinogenic conditions (Floyd, 1982; Thirunavukkarasu & Sakthisekaran, 2001). The observed decline in SOD and catalase activities in our study might be because of the increase in circulating lipid peroxides, which reportedly results in the accumulation of superoxide anions that are capable of traversing membranes causing deleterious effects at sites beyond the tumor. Glutathione peroxidase is reported to react with H_2O_2 to prevent the intracellular damage caused.

In the present study, the MNNG treatment significantly enhanced serum MDA level, whereas the level of serum GSH was decreased, when compared with the normal control mice. The treatment of TP extract (400, 600 and 800 mg/kg body weight) significantly enhanced serum GSH level, and decreased serum MDA level in a dose-dependent manner compared to model control group (Fig. 2).

The MNNG treatment significantly decreased stomach SOD, CAT and GSH-Px activities, when compared with the normal control mice. The treatment of TP extract (400, 600 and 800 mg/kg body weight) significantly enhanced stomach SOD, CAT and GSH-Px activities in a dose-dependent manner compared to model control group (Table 2). Our results proved that oxidative injury had happened in gastric cancer animals. Tea polysaccharides could significantly decrease lipid peroxidation level and increased antioxidant enzymes activities in gastric cancer animals.

An antibody, also known as an immunoglobulin, is a large Y-shaped protein produced by B-cells that is used by the immune system to identify and neutralize foreign objects such as bacteria and viruses. The antibody recognizes a unique part of the foreign target, called an antigen (Janeway, 2001; Alkahtani, AL-Farraj, Alarifi, Saad, & Al-Dahmash, 2011; Jain, Bhuiyan, Hossain, & Bachar, 2011; Yang, Toffa, Lohn, Seifalian, & Winslet, 2005). Antibodies are substances made by the body's immune system in response to bacteria, viruses, fungus, animal dander, or cancer cells. Antibodies attach to the foreign substances so the immune system can destroy them.

Table 3
Effect of tea polysaccharides on serum IgA, IgM and IgG levels.

Group	IgA	IgM	IgG
NC	13.91 ± 1.17	3.37 ± 0.28	1.72 ± 0.16
MC	6.44 ± 0.53**	1.51 ± 0.13**	1.09 ± 0.08**
TP (400 mg/kg b.w.)	8.91 ± 0.59±±	1.98 ± 0.15±	1.41 ± 0.11±±
TP (600 mg/kg b.w.)	10.77 ± 0.95±±	2.71 ± 0.22±±	1.58 ± 0.14±±
TP (800 mg/kg b.w.)	13.05 ± 1.18±±	3.29 ± 0.28±±	1.69 ± 0.15±±

** $P < 0.01$, compared with NC group.
± $P < 0.05$.
±± $P < 0.01$, compared with MC group.

Table 3 shows that the serum IgA, IgM and IgG levels were significantly lower in the MC group than in the NC group ($P < 0.01$). The three doses (400, 600 and 800 mg/kg body weight) of TP dose-dependently markedly enhanced serum IgA, IgM and IgG levels in the TP groups compared to MC group ($P < 0.05$, $P < 0.01$).

Cytokine is a small protein released by cells that has a specific effect on the interactions between cells, on communications between cells or on the behavior of cells. The cytokines includes the interleukins, lymphokines and cell signal molecules, such as tumor necrosis factor and the interferons, which trigger inflammation and respond to infections.

IL-10 produced by a wide variety of cell types, including T cells, dendritic cells, B cells, monocytes, macrophages, and mast cells, shows potent anti-inflammatory and immunosuppressive potential by inhibiting a broad spectrum of dendritic cell and macrophage functions, thus effectively limiting the ability of antigen presenting cells to activate T cells (Umetsu & Winandy, 2009; Gönenç, Hacışevki, Aslan, Torun, & Şimşek, 2012; Swamy et al., 2011; Kim, Kang, Gal, Cho, & Bahk, 2011). Furthermore, IL-10 is an important immunoregulatory cytokine that inhibits T cell function by suppressing the expression of proinflammatory cytokines (mostly Th1 cytokines), such as TNF-α, IL-1, IL-6, IL-8, and IL-12 (de Waal Malefyt et al., 1991).

Proinflammatory cytokines such as IL-1β, IL-6, TNF-α and GM-CSF act as autocrine growth factors for tumor angiogenesis. These cytokines could be prometastatic or proangiogenic and their deregulated expression directly correlates with the metastatic potential of several human carcinomas (Isner & Asahara, 1999; Obameso, Akinyele, Oladunmoye, & Osho, 2011; Polat, 2011; Kekec et al., 2009). Moreover altered levels of proinflammatory and proangiogenic factors are observed in various forms of cancer (Chen et al., 1999). Upregulation of these cytokines was closely linked to chronic inflammation and cancer (Aggarwal, Shishodia, Sandur, Pandey, & Sethi, 2006).

Table 4 shows that the serum IL-2, IL-4 and IL-10 levels were significantly lower in the MC group than in the NC group ($P < 0.01$). The three doses (400, 600 and 800 mg/kg body weight) of TP dose-dependently markedly enhanced serum IL-2, IL-4 and IL-10 levels in the TP groups compared to MC group ($P < 0.05$, $P < 0.01$).

The levels of serum IL-6 and TNF-α were significantly higher in MC mice than in normal control mice at 50 days after the onset of gastric cancer ($P < 0.01$). Administration of TP (400, 600 and 800 mg/kg body weight) for 50 days suppressed the increase in serum IL-6 and TNF-α levels in the gastric cancer mice to the same extent as NC ($P < 0.05$, $P < 0.01$) (Table 5).

Table 4
Effect of tea polysaccharides treatment on serum IL-2, IL-4 and IL-10 levels.

Group	IL-2 (ng/ml)	IL-4 (ng/ml)	IL-10 (ng/ml)
NC	7.08 ± 0.57	26.61 ± 1.92	85.59 ± 5.84
MC	5.15 ± 0.42**	17.82 ± 1.63**	60.81 ± 4.63**
TP (400 mg/kg b.w.)	5.93 ± 0.39±	20.71 ± 1.88±±	70.53 ± 5.77±±
TP (600 mg/kg b.w.)	6.38 ± 0.51±±	23.62 ± 1.78±±	78.49 ± 5.09±±
TP (800 mg/kg b.w.)	7.24 ± 0.63±±	25.55 ± 2.11±±	83.51 ± 4.82±±

** $P < 0.01$, compared with NC group.
± $P < 0.05$.
±± $P < 0.01$, compared with MC group.

Table 5
Effect of tea polysaccharides treatment on serum IL-6 and TNF-α levels.

Group	IL-6 (ng/ml)	TNF-α (ng/ml)
NC	80.17 ± 7.03	3.09 ± 0.27
MC	99.05 ± 6.83**	4.72 ± 0.41**
TP (400 mg/kg b.w.)	92.03 ± 5.66±	4.11 ± 0.35±
TP (600 mg/kg b.w.)	87.59 ± 7.11±±	3.72 ± 0.29±±
TP (800 mg/kg b.w.)	79.39 ± 5.81±±	3.22 ± 0.28±±

** $P < 0.01$, compared with NC group.
± $P < 0.05$.
±± $P < 0.01$, compared with MC group.

4. Conclusion

In this study, we evaluated the protective effects of TP on gastric cancer mice. Oxidative stress induced by MNNG was attenuated by treatment with TP, as examined by measuring the levels of SOD, CAT, GSH-Px and lipid peroxidation. Furthermore, TP treatment significantly decreased the levels of pro-inflammatory cytokines such as TNF-α, and IL-6 and increased the level of anti-inflammatory cytokines. Taken together, these results suggest that treatment of mice with TP can significantly attenuate the oxidative damage and inflammation induced by MNNG.

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