

## Inhibitory Effect of Capsaicin against Carcinogen-induced Oxidative Damage in Rats

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### Abstract

Capsaicin (trans-8-methyl-N-vanillyl-6-nonenamide), a major pungent component of hot pepper, is known to exert antioxidative properties. In this study, we investigated the protective effects of capsaicin against chemical carcinogen-induced oxidative damage in rats. Male Sprague Dawley rats weighting 230~250 g were treated with chemical carcinogens such as 2-nitropropane (2NP) or n-methyl-N'-nitro-N-nitrosoguanidine (MNNG) after (or before) the administration of capsaicin at doses of 0.5, 1, 5 mg/kg. The level of lipid peroxidation in rat liver was estimated by measuring the amounts of thiobarbituric acid reactive substances. The degree of oxidative DNA damage was evaluated by measuring a DNA adduct, 8-hydroxydeoxyguanosine (8-OHdG), in urine. Antioxidative activities of capsaicin and its metabolites *in vitro* were determined by the measurement of DPPH (1,1-diphenyl-2-picrylhydrazyl), a radical quencher. Significant inhibition of 2-NP induced lipid peroxidation was observed in the liver of the rat when treated with capsaicin. MNNG-induced urinary excretion of 8-OHdG was decreased by capsaicin treatment. Capsaicin and its metabolites inhibited not only the formation of free radicals, but also lipid peroxidation *in vitro*. Our results show that capsaicin may function as a free radical scavenger against chemical carcinogen-induced oxidative cellular damage *in vivo*. The observed antioxidative activities of capsaicin may play an important role in the process of chemoprevention.

**Key words:** capsaicin, carcinogen, 2-nitropropane, n-methyl-N'-nitro-N-nitrosoguanidine, oxidative damage, thiobarbituric acid reactive substances, 8-hydroxydeoxyguanosine, free radicals, scavenging activity, chemoprevention

### INTRODUCTION

Capsaicin (trans-8-methyl-N-vanillyl-6-nonenamide) is a major pungent principle in hot pepper, which is widely consumed as a spice and food additive in Korea and other countries. The intake of excessively hot peppers has been considered to be a risk factor for gastrointestinal pathologies, particularly gastric cancers. Recent reports, however, showed that capsaicin had a beneficial effect on gastric mucosal injury or certain types of cancers (1-5). Capsaicin inhibits a cytochrome P450 2E1, an isoform that catalyzes metabolic activation of various chemicals such as tobacco-specific nitrosamines, benzo[a]pyrene, and aflatoxins (5-7). The metabolic inactivation induced by capsaicin elicits the antimutagenic and anticarcinogenic properties (7). Capsaicin-induced apoptotic cell death has been reported in stomach cancer, hepatocarcinoma and neuroblastoma cells (8,9), and inhibited the growth of a number of transformed cell lines (10). Our recent study has demonstrated

that capsaicin inhibits n-methyl-N'-nitro-N-nitrosoguanidine (MNNG)-induced gastric carcinogenesis by altering the tumor forming-related gene expression (11). These findings suggest that capsaicin may play a protective role against the pathological process of carcinogenesis.

Most chemical carcinogens generate the oxygen free radicals through their metabolic activation process. The activated oxygen free radicals cause oxidative cellular injury to membrane, protein, and DNA. This process is strongly associated with the basic pathological phenomena in the initiation, promotion, and progression of carcinogenesis (12,13). Therefore, antioxidants and oxygen free radical scavengers are considered to be potential drug candidates for chemoprevention in cellular and molecular aspects. Our previous study has demonstrated that capsaicin inhibits *in vitro* nitrosation in a similar way to alpha-tocopherol (14), indicating that capsaicin may be protective against carcinogen-induced cellular damage. Capsaicin is known to elicit antioxidative activity (15-18). However,

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it has not been known whether capsaicin exerts chemopreventive properties by the antioxidative activity. Since antioxidants is considered to be chemopreventive, we hypothesized that capsaicin could exert chemopreventive properties by inhibiting oxidative cellular damage *in vivo*. In the present study, the protective effect of capsaicin against carcinogen-induced oxidative damage was investigated by the evaluation of lipid peroxidation, a DNA adduct formation, and free radical scavenging activities *in vivo* and/or *in vitro*. Our data suggest that the antioxidative activity of capsaicin may be associated with its chemopreventive properties.

## MATERIALS AND METHODS

### Animal treatment

Male SD rats weighting 230~250 g were housed in a temperature controlled room (22°C) under 12-h light-dark cycle (8:00 pm to 8:00 am) and had free access to tap water and standard laboratory chow (Samyang Purina Inc, Korea). The care and treatment of experimental animals conformed to the NIH guidelines for the ethical treatment of laboratory animals. We performed the experiments with the least possible pain or discomfort to the animals. Rats were fasted for 18 h but had free access to water before the experiment. Capsaicin was dissolved 10% ethanol, 10% Tween 80 (Sigma), and 80% 0.15 N sodium chloride. The animals were treated with 2-nitropropane (2NP, 500 mg/kg BW) or *n*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG, 100 mg/kg BW) after and/or before the administration of capsaicin at doses 0.5, 1, 5 mg/kg, BW or vehicle alone.

### Determination of lipid peroxidation

2NP-induced lipid peroxidation was estimated in liver by measuring thiobarbituric acid reactive substances (TBARS). To prepare microsome, liver (1 g) was homogenized in 1.15% KCl and was centrifuged at 1,200 rpm for 10 min at 4°C. The supernatant was centrifuged at 15,000 rpm for 30 min at 4°C and was used for detecting lipid peroxidation. The reaction mixture contained 0.1 mL of sample, 0.2 mL of 8.1% sodium dodecyl sulfate, 1.5 mL of 20% acetic acid solution of pH 3.0, and 1.5 mL of 0.8% aqueous solution of TBA. The mixture was finally made up to 4.0 mL with distilled water and heated at 95°C for 60 min. After cooling with tap water, 1.0 mL of distilled water and 5.0 mL of the mixture of *n*-butanol and pyridine (15:1, v/v) were added, and the mixture was shaken vigorously. After centrifugation at 3000 rpm for 10 min, 1,1,3,3-Tetramethoxypropane was used as a standard for thiobarbituric acid reactive substances. The protein level was determined, and malondialdehyde (MDA) formed was estimated at 535 nm using extinction coefficient of  $1.56 \times 10^5 \text{ M}^{-1}\text{cm}^{-1}$  (19).

### Measurement of 8-OHdG in urine

MNNG-induced oxidative damage was estimated by measuring 8-OHdG in urine. Urinary 8-OHdG/creatinine levels of rats treated with MNNG were evaluated by enzyme-linked immunosorbent assay using a monoclonal antibody (20). All measurements were carried out according to the manufacture's instruction (Niken Food Inc, Japan).

### Measurement of free radical scavenging activity

Free radical scavenging activities of capsaicin and its metabolites were evaluated by measuring diphenylpicrylhydrazine (DPPH), a purple-colored stable free radical, which is reduced to yellow-color by antioxidants or reducing agents. DPPH (0.016%) in ethanol and sodium acetate buffer (pH 5.6) was prepared and mixed 1:1 (v/v) with the sample solution containing either linolic acid or various concentrations of capsaicin or its metabolites such as vanillyl amine, vanillyl alcohol, and vanillin. The absorbance was measured at 528 nm for 40 min (21).

### Statistics

Statistical analysis was performed using ANOVA and Student's *t* test. Pairwise comparisons were made using the Bonferroni inequality test. Differences were considered to be significant when  $p < 0.05$ .

## RESULTS AND DISCUSSION

The protective effect of capsaicin on carcinogen-induced cellular damage was investigated by using 2NP and MNNG. 2NP, a compound generating intermediate peroxy radicals by reducing  $\text{O}_2$  to  $\text{O}_2^-$ , is known to induce lipid peroxidation and act as a hepatocarcinogen (22). The inhibitory effect of capsaicin against 2NP-induced lipid peroxidation in liver of rats significantly ( $p < 0.01$ ) decreased in the group treated with capsaicin (0.5 and 5 mg/kg) compared to the control (Fig. 1). The carcinogenic activity of MNNG, which induces gastric carcinoma, is involved in the formation of DNA adducts (23,24). Namely 8-OHdG, an oxidatively modified nucleotide by oxygen free radicals, is known to increase in carcinogen-induced carcinogenesis (24-26). When MNNG-induced urinary secretion of 8-OHdG was measured in urine of rats, a moderate decrease of 8-OHdG excretion ( $p < 0.05$ ) was observed in the group treated with capsaicin compared to the control (Fig. 2), indicating that capsaicin ameliorated the MNNG-induced DNA damage in rat.

Naturally occurring antioxidants such as polyphenol or curcumin are capable of scavenging free radicals, and these scavenging activities are associated with the chemopreventive property. Particularly curcumin, which has structural similarity to capsaicin, has shown the inhibition activity of TPA-, benzo(a)pyrene-, azoxymethane-, *N*-ethyl-

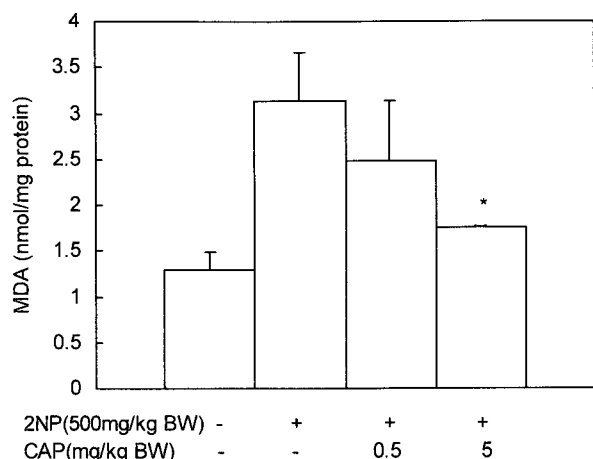


Fig. 1. Inhibitory effect of capsaicin against 2NP-induced lipid peroxidation in rats. The animals were treated with 2NP before the administration of capsaicin at doses 0.5, 5 mg/kg, BW or vehicle alone. Values are the mean  $\pm$  SEM (n=6). \*Significantly different from 2NP-treated group without capsaicin,  $p < 0.01$ .

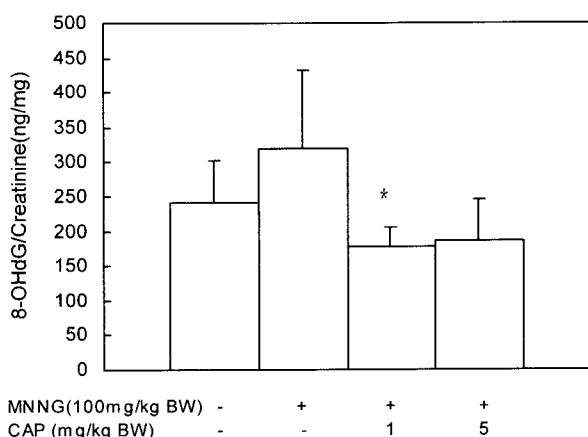


Fig. 2. Inhibitory effect of capsaicin against MNNG-induced urinary 8-OHdG production in rats. The animals were treated with MNNG after the administration of capsaicin at doses 1, 5 mg/kg, BW or vehicle alone. Values are the mean  $\pm$  SEM (n=6). \*Significantly different from MNNG-treated group without capsaicin,  $p < 0.05$ .

N'-nitro-N-nitrosoguanidine-induced gastrointestinal tumorigenesis in mice (27). Alpha-tocopherol has also been shown to inhibit MNNG-induced gastric carcinogenesis and mutagenesis (28,29). A recent report showed that capsaicin inhibited a mouse skin tumorigenesis induced with 12-O-tetradecanoyl phorbol-13-acetate (TPA) (30) by accelerating the production of  $O_2^-$  and  $H_2O_2$ , leading to oxidative cellular damage. Capsaicin metabolizes into vanillyl amine, vanillin, vanillyl alcohol, vanillic acid *in vivo* (31). Our previous study demonstrated that capsaicin and one of the metabolites vanillyl alcohol inhibited *in vitro* nitrosation, which is estimated by the formation of nitro-

soproline, as similar to alpha-tocopherol (14). These findings suggest that the inhibitory activity of capsaicin *in vivo* against the chemical carcinogen-induced oxidative cellular damage may possibly be associated with the scavenging activity of capsaicin and the metabolites.

To find whether the scavenging activities of capsaicin and the metabolites are implicated in its chemopreventive property, the level of free radical formation in the presence of capsaicin and its metabolites was investigated. As shown in Fig. 3, capsaicin exerted free radical scavenging activity in a dose dependent manner in linoleic acid oxidation model system. Capsaicin and vanillyl alcohol inhibited the free radical formation by 50~60% of butylated hydroxyanisole (BHA) or alpha-tocopherol activity, while vanillyl amine and vanillic acid inhibited by 25~30% (Fig. 4). The inhibitory activity of capsaicin *in vitro* lipid peroxidation was very similar to the one of BHA and alpha tocopherol (Fig. 5), well-known scavengers of peroxy radicals and inhibitor of chemical carcinogenesis (32-35). These findings support the idea that capsaicin and its metabolites act as a free radical scavenger and thereby play an important role for the chemopreventive properties. In addition, capsaicin is a phenolic compound like curcumin, and the phenolic and methoxy group of capsaicin might contribute to its free radical scavenging activity, resulting in its antioxidant properties of against oxidative damage.

In conclusion, capsaicin exerts a protective effect against 2NP-induced lipid peroxidation and MNNG-induced 8-OHdG formation in rats. The antioxidative activity against the carcinogen-induced oxidative cellular damage

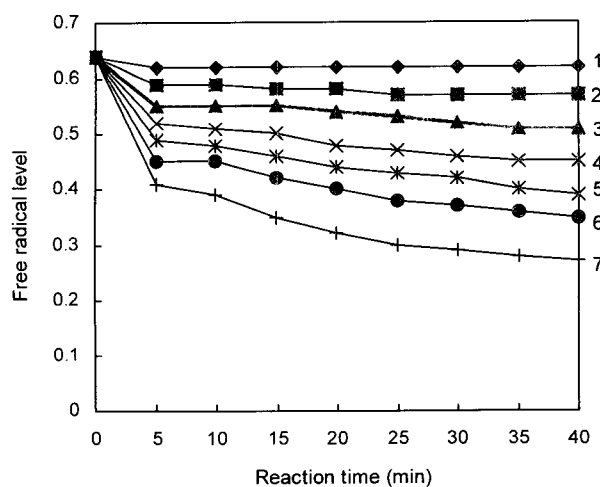
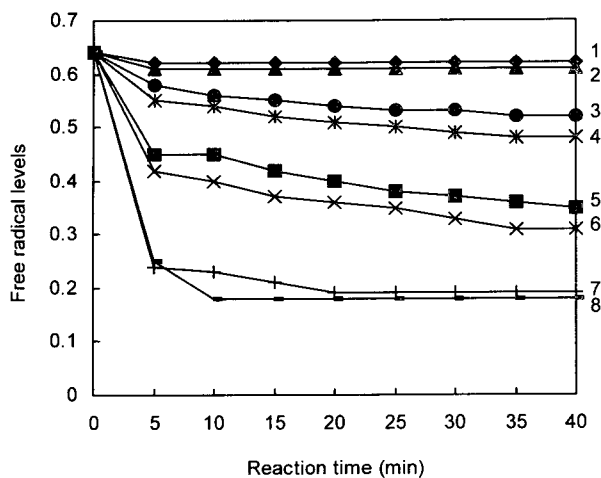
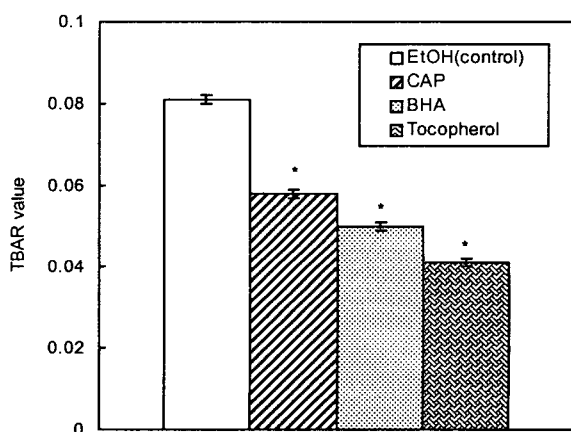


Fig. 3. Free radical scavenging activity of capsaicin *in vitro*. Increasing dose of capsaicin (100~1000  $\mu$ M) was added to the reaction mixture and the change of absorbance was measured at various time points. 1. Ethanol (control) 2. Capsaicin 100  $\mu$ M 3. Capsaicin 200  $\mu$ M 4. Capsaicin 300  $\mu$ M 5. Capsaicin 400  $\mu$ M 6. Capsaicin 500  $\mu$ M 7. Capsaicin 1000  $\mu$ M.



**Fig. 4.** Free radicals scavenging activity of capsaicin and its metabolites *in vitro*. The reaction mixture contains 500  $\mu$ M of capsaicin, its metabolites, BHA, or alpha-tocopherol. 1. Ethanol (control) 2. Vanillin 3. Vanillyl amine 4. Vanillic acid 5. Capsaicin 6. Vanillyl alcohol 7. BHA 8. Alpha-tocopherol. The reaction was performed as described in Materials and Methods.



**Fig. 5.** Inhibitory activity of capsaicin on lipid peroxidation *in vitro*. Liver microsome was prepared as described in Materials and Methods. The reaction mixture contains 100  $\mu$ M of capsaicin, BHA, or alpha-tocopherol. The reaction was performed as described in Materials and Methods. Values are the mean SEM. \*Significantly different from control,  $p < 0.01$ .

may be an important factor to understand its chemopreventive property.

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