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Chemoprevention of 4-Nitroquinoline 1-Oxide-induced Rat Oral Carcinogenesis by the Dietary Flavonoids Chalcone, 2-Hydroxychalcone, and Quercetin[1]

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ABSTRACT

The modifying effects of dietary exposure of three flavonoids, chalcone, 2-hydroxychalcone, and quercetin, during the initiation and postinitiation phases of oral tumorigenesis initiated with 4-nitroquinoline-1-oxide (4-NQO) were investigated in male F344 rats. At 6 weeks of age, animals were divided into experimental and control groups. At 7 weeks of age, all animals except those treated with test chemicals alone and the untreated control group were given 4-NQO [20 parts/million (ppm)] in the drinking water for 8 weeks to induce oral neoplasms. For chemopreventive study by feeding of test compounds during the initiation phase, groups of animals were given diets containing 500 ppm chalcone, 500 ppm 2-hydroxychalcone, or 500 ppm quercetin for 10 weeks, starting 1 week before 4-NQO exposure. Seven days after stopping 4-NQO exposure, these groups were switched to the basal diet and kept on this diet until the end of the experiment. For chemopreventive study by treatment with test chemicals during the postinitiation phase, starting 1 week after the cessation of 4-NQO administration, the groups given 4-NQO and the basal diet were switched to the diets mixed with test chemicals and maintained on these diets for 22 weeks. The other groups consisted of rats fed diets containing 500 ppm test chemicals alone or of untreated rats. Thirty-two weeks after the start of the study, the incidence of tongue neoplasms and preneoplastic lesions, polyamine levels in the tongue epithelium, and cell proliferation activity estimated by bromodeoxyuridine labeling index were compared among the different dietary groups. Feeding of all test chemicals during either initiation or postinitiation phases caused a significant reduction in the frequency of tongue carcinoma (68–88% reduction; $P < 0.05$). Dietary administration of these test chemicals also significantly decreased the bromodeoxyuridine labeling index of the tongue squamous epithelium ($P < 0.05$). In addition, polyamine levels in the oral mucosa were lowered in rats treated with 4-NQO and test chemicals when compared to those given 4-NQO alone. These results indicate that the flavonoids chalcone, 2-hydroxychalcone, and quercetin present in our daily foods have an inhibitory effect on oral carcinogenesis initiated with 4-NQO, and such a modifying effect may be related partly to the suppression of cell proliferation.

INTRODUCTION

Tobacco and alcohol use are the major risk factors in the development of oral cancer. Also, combining exposure to tobacco and alcohol results in an increased cancer incidence (synergistic effect rather than additive effect; Refs. 1–3). It is well known that survival of patients with head and neck cancers, including oral cancer, has not improved significantly, despite recent advances in radiotherapy and/or chemotherapy. Furthermore, a significant number of patients cured by primary treatment will develop a second cancer within a few years, usually in the head and neck region or the lung or esophagus. Many people using the cocarcinogens of tobacco and alcohol increase their risk of developing simultaneous or subsequent second primary epithelial cancers of these regions (4–6). The concept that common carcinogen exposure affects the entire epithelial lining of the lungs and upper aerodigestive tract is termed "field cancerization" (7, 8). It is necessary to embrace bright new ideas that will change the prognosis of this malignancy. One such promising approach is chemoprevention (9). Chemopreventive agents include chemicals from nontoxic natural substances or synthetic chemicals (10, 11). Retinoids have been reported to be effective against chemical carcinogenesis in lungs and upper aerodigestive tracts of animal models (12–14), and some retinoids are candidates for chemopreventives in human cancer development (15, 16). On the basis of currently available evidence, retinoids are considered to be a representative chemopreventive agent. However, toxicity such as skin dryness, cheilitis, hypertriglyceridemia, and conjunctivitis associated with retinoids at doses used in these studies limits their potential for chemoprevention. For this reason, other agents (natural or synthetic) that retain their chemopreventive activity but are less toxic have been sought and tested.

The oral cancer may start from hyperplastic (nondysplastic) and progress to invasive carcinoma through dysplasia and carcinoma *in situ*, and this process serves as a good model to investigate multistep oral carcinogenesis (17–19). Moreover, in terms of its easy accessibility for examination and follow-up of neoplastic changes, the oral cavity is an excellent target organ for experimental chemoprevention studies (19). The most widely studied oral epithelial tumor model is the experimental squamous cell carcinoma of the hamster buccal pouch with DMBA.[3] Hamster buccal pouch lesions and human lesions seem to be similar in the histological features of dysplasia. However, the cheek pouch has no anatomic counterpart in humans, and this epithelium is considerably thinner in the other parts of the oral mucosa. Thus, the DMBA hamster buccal pouch model has several features that are not in common with humans (20). 4-NQO, a water-soluble quinoline derivative, produces a spectrum of preneoplastic and neoplastic lesions in the oral cavity, especially tongue, of rats following application by drinking water with 4-NQO. Thus, 4-NQO-induced lesions appear to have significant advantages when compared to DMBA-induced hamster buccal pouch lesions. Using animal models of 4-NQO-induced oral carcinogenesis for experimental chemoprevention, we have reported some candidates without toxicity against oral malignancy (21–29). These include minor non-nutrients (19).

Flavonoids are benzo-γ-pyrone derivatives and are distributed widely in the plant kingdom. Some flavonoids have been known to exert a wide variety of biological actions, such as antiallergic, anti-inflammatory, and anticarcinogenic, on mammalian systems (30, 31). They are found commonly in the human diet and are estimated to be consumed in a quantity of approximately 1 g/day. (32). Quercetin (3,3′,4′,5,7-pentahydroxyflavone) is one of the most abundant natural flavonoids, being present in various common vegetables and fruits (32). Quercetin has been reported to be one of most potent mutagens in the Ames test and other short-term *in vitro* tests (33–35). Although initial studies reported that quercetin causes an increase in the incidence of intestinal, urinary bladder, and liver tumors in *in vivo* tests

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[3] The abbreviations used are: DMBA, 9,10-dimethyl-1,2-benzanthracene; 4-NQO, 4-nitroquinoline 1-oxide; BrdUrd, bromodeoxyuridine; ppm, parts/million.

using rats (36, 37), subsequent *in vitro* studies suggest that quercetin exerts powerful growth-inhibitory activity on the human squamous cell carcinoma cell line of the tongue and other human cancer cell lines of various organs (38–43). A dietary supplement of quercetin inhibits the development of carcinogen-induced rat mammary cancer (44) and colonic neoplasia (45). Moreover, a topical application of quercetin inhibited skin tumor formation (46, 47).

Other flavonoids, including chalcones, are also abundantly present in nature, from ferns to higher plants. They are of the stilbene configuration with the bracketing of an α,β-unsaturated carbonyl group by two phenyl groups. Some chalcones have also been reported to possess anti-inflammatory, analgesic, antipyretic, bactericidal, and antifungal properties (48). Some derivatives of chalcones are reported to be antimutagenic and to have inhibitory effects on human cancer cell proliferation in *in vitro* tests (48–52). Moreover, in *in vivo* studies, some chalcones could inhibit carcinogen-induced pulmonary and mammary carcinogenesis by dietary administration and 12-*O*-tetradecanoylphorbol-13-acetate-induced skin tumor promotion by topical application using animal models (49, 53–55).

In the current study, we evaluated the modifying effects of the dietary flavonoids chalcone, 2-hydroxychalcone, and quercetin (Fig. 1) during the initiation and postinitiation phases on 4-NQO-induced oral carcinogenesis in rats. The effects of these flavonoids on expression of the proliferation biomarkers such as polyamine levels and BrdUrd labeling index were assessed to clarify the underlying mechanism(s) of modification.

MATERIALS AND METHODS

Animals, Diets, Test Chemicals, and Carcinogen. Male F344 rats, 4 weeks old, were purchased from Japan SLC, Inc. (Hamamatsu City, Japan). The rats were housed in a holding room under the controlled conditions of a 12-h light/dark cycle, 23 ± 2°C temperature, and 50 ± 10% humidity. After 2 weeks of quarantine, the rats were randomized into experimental and control groups. They were housed three or four to a wire cage. Food and water were available *ad libitum*. Powdered CE-2 (CLEA Japan, Inc., Tokyo, Japan) was used as a basal diet during the experiment. It contained 50.4% crude carbohydrate, 24.8% crude protein, 4.6% crude fat, 7.2% ash, 4.2% crude cellulose, and 8.8% water but did not contain any compounds present in the plant food. 4-NQO, chalcone, and 2-hydroxychalcone were obtained from Tokyo Kasei Organic Chemicals Co., Ltd. (Tokyo, Japan). Quercetin dihydrate was obtained from Fluka Fine Chemicals (Tokyo, Japan). These flavonoids for dietary administration were each blended into a powdered basal diet at a dose of 500 ppm and stored in a dark, cold room (4°C). 4-NQO was dissolved in tap water to a final concentration of 20 ppm and stored in a dark, cold room.

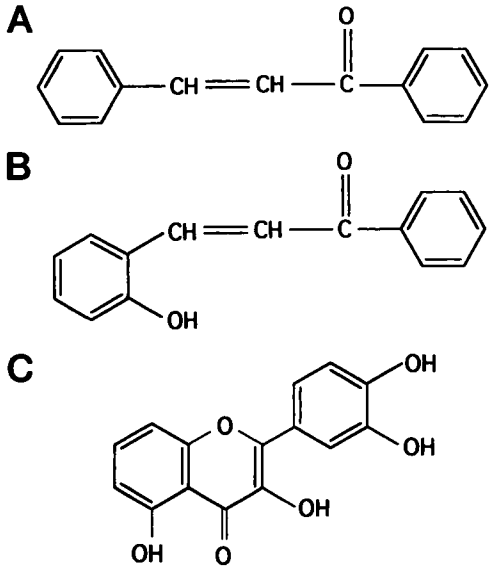

Fig. 1. Chemical structures of test chemicals. *A*, chalcone; *B*, 2-hydroxychalcone; *C*, quercetin.

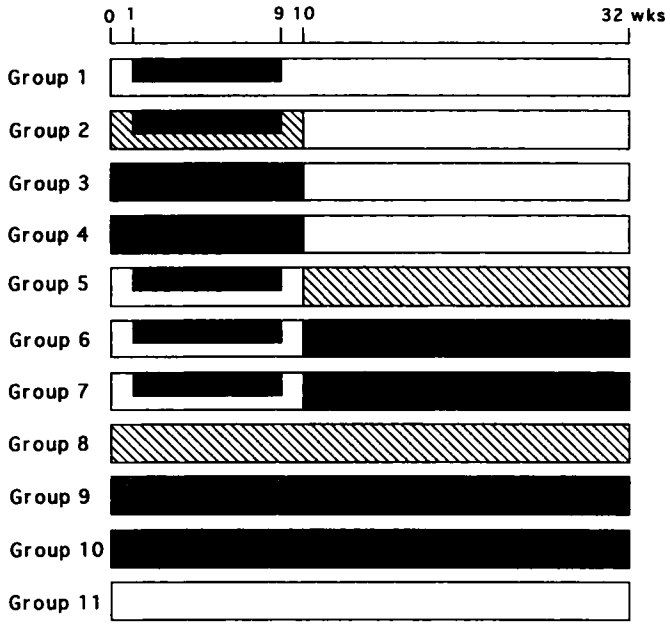

Fig. 2. Experimental protocol. ■, 4-NQO, 20 ppm in drinking water; ▨, 500 ppm chalcone in diet; ▧, 500 ppm 2-hydroxychalcone in diet; ■, 500 ppm quercetin in diet; □, basal diet and tap water.

Experimental Procedures. A total of 140 rats was divided into 11 groups as shown in Fig. 2. At 7 weeks of age, rats in groups 1–7 were given 20 ppm 4-NQO in drinking water for 8 weeks. Starting at 6 weeks of age, groups 2, 3, and 4 were fed the diets containing, respectively, chalcone, 2-hydroxychalcone, and quercetin at a dose of 500 ppm for 10 weeks and then switched to the basal diet and kept on the diet for 22 weeks. Groups 5, 6, and 7 were fed the diet containing, respectively, chalcone, 2-hydroxychalcone, and quercetin at a dose of 500 ppm starting 1 week after the cessation of 4-NQO treatment and kept on these diets for 22 weeks. Groups 8, 9, and 10 were fed the diets mixed with the above respective test chemicals alone at a dose of 500 ppm throughout the experiment. Group 11 was given the basal diet without test chemicals and tap water without 4-NQO throughout the experiment and served as an untreated control. All rats were carefully observed daily, and consumption of the drinking water containing 4-NQO or the diets mixed with test chemicals was recorded to estimate intake of the chemicals. The experiment was terminated at 32 weeks, and all animals were killed. At necropsy, all organs, especially the oral cavity, were carefully inspected to find the preneoplastic and neoplastic lesions. Tongues were cut into approximate halves; one portion was used for the polyamine assay and the other for histopathology and counts of cell proliferation biomarkers. For histological confirmation, tissue and gross lesions were fixed in 10% buffered formalin and embedded in paraffin blocks, and the sections were stained by H&E. Epithelial lesions (hyperplasia, dysplasia, and neoplasia) in the oral cavity were diagnosed according to the criteria described by Bánóczy and Csiba (56) and WHO (57).

Polyamine Levels of Tongue Tissue. The polyamine levels in the oral cavity tissues were determined by the method of Koide *et al.* (58). The results obtained correlated well with those measured by high-performance liquid chromatography. When the animals were killed, half of the tongues of all rats were collected, and the amounts of diamine, spermine, and spermidine and the sum of these were determined by an enzymatic differential assay.

Determination of Proliferative Activity in the Tongue Epithelium by BrdUrd Labeling Index. To assess the proliferative activity of squamous epithelium of the tongue, the BrdUrd labeling indices of all animals were quantified. For measurement of BrdUrd-incorporated nuclei, all animals were given an i.p. injection of 50 mg/kg body weight BrdUrd (Sigma Chemical Co., St. Louis, MO) 1 h prior to killing. One half of the tongue was used, and two serial sections were made after embedding in paraffin. One section was used

Table 1 *Body, liver, and relative liver weights in each group*

Group	Treatment	No. of rats examined	Body weight (g)	Liver weight (g)	Relative liver weight (g/100 g body weight)
1	4-NQO alone	15	352 ± 23[a]	12.8 ± 1.5	3.65 ± 0.36
2	4-NQO + chalcone (500 ppm)	16	335 ± 15[b]	13.3 ± 1.3	3.97 ± 0.42[b]
3	4-NQO + 2-hydroxychalcone (500 ppm)	16	356 ± 18	14.2 ± 1.3[c]	4.02 ± 0.44[c]
4	4-NQO + quercetin (500 ppm)	16	342 ± 20	12.8 ± 1.0	3.75 ± 0.27
5	4-NQO → chalcone (500 ppm)	16	326 ± 16[c]	13.2 ± 1.1	4.06 ± 0.28[c]
6	4-NQO → 2-hydroxychalcone (500 ppm)	16	332 ± 18[c]	12.7 ± 1.5	3.82 ± 0.42
7	4-NQO → quercetin (500 ppm)	15	343 ± 25	14.4 ± 2.3[b]	4.20 ± 0.60[c]
8	Chalcone (500 ppm)	8	320 ± 20[d]	12.1 ± 1.4	3.79 ± 0.26
9	2-Hydroxychalcone (500 ppm)	8	327 ± 17[d]	12.6 ± 1.6	3.83 ± 0.31
10	Quercetin (500 ppm)	6	329 ± 23	11.5 ± 1.9[d]	3.48 ± 0.41[d]
11	No treatment	8	343 ± 18	13.2 ± 1.2	3.83 ± 0.24

[a] Mean ± SD.
[b] Significantly different from group 1 by Dunnett's t test ($P < 0.05$).
[c] Significantly different from group 1 by Dunnett's t test ($P < 0.01$).
[d] Significantly different from group 11 by Dunnett's t test ($P < 0.05$).

for histopathology, and the other was used for the immunohistochemical detection of BrdUrd incorporation using an immunohistochemical analysis kit (DAKO Japan, Kyoto, Japan). The labeling indices of BrdUrd (percentages) were calculated by counting the labeled nuclei of 100 cells in normal or nonlesional tongue epithelium of each rat.

Statistical Analysis. Statistical analysis on the incidence of lesions was performed using Fisher's exact probability test or χ^2 test, and the data from measurements of body and liver weight, the data from the polyamine assay, and BrdUrd labeling index were compared by Dunnett's t test. The results were considered statistically significant if the P value was 0.05 or less.

RESULTS

General Observations. Animals in groups 1–7 tolerated well the oral administration of 4-NQO and/or test chemicals. Food intake for test chemical treatment groups was 15.5–16.5 g/day/animal (test chemical intake was 7.8–8.3 mg/day/animal). There were no significant differences in the total intake of 4-NQO/rat among the seven groups (data not shown). The mean body, liver, and relative liver weights at the end of the study are indicated in Table 1. The mean body and liver weights of rats given the diet containing test chemicals alone (groups 8–10) were lower than those of the control group (group 11). Mean body weights in groups 2, 5, and 6 were significantly smaller than those of group 1 ($P < 0.05$, $P < 0.01$, and $P < 0.01$, respectively). Mean liver weights in groups 3 and 7 were significantly larger than those of group 1 ($P < 0.05$, and $P < 0.01$, respectively). Average relative liver weights in groups 2, 3, 5, and 7 were significantly larger than in group 1 ($P < 0.05$, $P < 0.01$, $P < 0.01$, and $P < 0.01$, respectively). Mean body weights in groups 8 and 9 were significantly smaller than those of group 11 ($P < 0.05$). Mean liver weights in group 10 were significantly smaller than those of group 11 ($P < 0.05$). Average relative liver weight in group 10 was significantly smaller than group 11 ($P < 0.05$). In this study, dietary administration of these test chemicals caused no clinical signs of toxicity, low survival rates, poor conditions, or histological changes suggesting toxicity in the liver, kidney, and lung.

Incidence of Tumors and Preneoplastic Lesions. In this study, endophytic and exophytic tumors developed only in the oral cavity, especially the dorsal site of the tongue of rats in groups 1–7. The former were mainly histologically well-differentiated squamous cell carcinomas, and the latter were squamous cell papillomas. Animals in groups 8–11 did not have any preneoplastic or neoplastic lesions. The incidence of tumors (squamous cell papillomas and carcinomas) in each group is shown in Table 2. In group 1 (4-NQO alone), the incidence of tongue squamous cell carcinoma and that of squamous cell papilloma were 60 and 27%, respectively. On the other hand, only a few rats (7–19%) either given test chemicals during 4-NQO administration (groups 2–4) or fed test chemicals after 4-NQO exposure (group 5–7) had tongue neoplasms. Statistical analysis revealed a significant decrease in the incidence of tongue carcinoma in rats fed test chemicals during the initiation (groups 2–4) or postinitiation phase (groups 5–7) when compared with that of group 1 ($P < 0.05$). Besides these neoplasms, a number of hyperplasia or dysplasia, which are considered to be preneoplastic lesions for oral cancer, were also present in the tongue of rats in group 1–7. The incidence of such lesions is shown in Table 2. The incidence of total hyperplasia and total dysplasia in groups 2–7 was lower than that in group 1. Such reduction in the incidence of preneoplastic lesions in rats fed test chemicals during the initiation (groups 2–4) or postinitiation phase (groups 5–7) was comparable. As for dysplasia with various degrees, reduction in the frequency of severe dysplasia was prominent (68% reduction in groups 2, 5, and 6; 78% reduction in groups 3 and 4; and 55% reduction in group 7, as shown in Table 3).

Tissue Polyamine Levels. The results of the polyamine assay of tongue epithelium are indicated in Table 4. Total polyamine (dia-

Table 2 *Incidence of tongue preneoplasms and neoplasms in rats of each group*

Group	Treatment	No. of rats examined	Hyperplasia (%)	Dysplasia (%)	Papilloma (%)	Squamous cell carcinoma (%)
1	4-NQO alone	15	15 (100)	13 (87)	4 (27)	9 (60)
2	4-NQO + chalcone (500 ppm)	16	10 (63)[a]	11 (69)	1 (6)	3 (19)[a]
3	4-NQO + 2-hydroxychalcone (500 ppm)	16	10 (63)[a]	8 (50)[a]	1 (6)	2 (13)[a]
4	4-NQO + quercetin (500 ppm)	16	11 (69)[a]	8 (50)[a]	1 (6)	2 (13)[a]
5	4-NQO → chalcone (500 ppm)	16	13 (81)	11 (69)	0 (0)[a]	3 (19)[a]
6	4-NQO → 2-hydroxychalcone (500 ppm)	16	11 (69)[a]	9 (56)	0 (0)[a]	3 (19)[a]
7	4-NQO → quercetin (500 ppm)	15	10 (67)[a]	12 (80)	1 (7)	1 (7)[a]
8	Chalcone (500 ppm)	8	0	0	0	0
9	2-Hydroxychalcone (500 ppm)	8	0	0	0	0
10	Quercetin (500 ppm)	6	0	0	0	0
11	No treatment	8	0	0	0	0

[a] Significantly different from group 1 by Fisher's exact probability test ($P < 0.05$).

Table 3 *Incidence of tongue preneoplasms in rats of each group*

Group	Treatment	Hyperplasia (%)			Dysplasia (%)			
		Total	Simple	Papillary	Total	Mild	Moderate	Severe
1	4-NQO alone	15 (100)	15 (100)	11 (73)	13 (87)	1 (7)	6 (40)	9 (60)
2	4-NQO + chalcone (500 ppm)	10 (63)[a]	10 (63)[a]	5 (31)[a]	11 (69)	4 (25)	5 (31)	3 (19)[a]
3	4-NQO + 2-hydroxychalcone (500 ppm)	10 (63)[a]	10 (63)[a]	2 (13)[a]	8 (50)[a]	4 (25)	3 (19)	2 (13)[a]
4	4-NQO + quercetin (500 ppm)	11 (69)[a]	11 (69)[a]	6 (38)[a]	8 (50)[a]	2 (13)	5 (31)	2 (13)[a]
5	4-NQO → chalcone (500 ppm)	13 (81)	13 (81)	6 (38)[a]	11 (69)	3 (19)	6 (38)	3 (19)[a]
6	4-NQO → 2-hydroxychalcone (500 ppm)	11 (69)[a]	11 (69)[a]	7 (44)	9 (56)	3 (19)	3 (19)	3 (19)[a]
7	4-NQO → quercetin (500 ppm)	10 (67)[a]	10 (67)[a]	2 (13)[a]	12 (80)	3 (20)	6 (40)	4 (27)
8	Chalcone (500 ppm)	0	0	0	0	0	0	0
9	2-Hydroxychalcone (500 ppm)	0	0	0	0	0	0	0
10	Quercetin (500 ppm)	0	0	0	0	0	0	0
11	No treatment	0	0	0	0	0	0	0

[a] Significantly different from group 1 by Fisher's exact probability test ($P < 0.05$).

mine + spermidine + spermine), spermidine, and spermine levels in rats given 4-NQO exposure (group 1) were greater than in nontreated rats (group 11). Total polyamine levels and the amounts of spermine in groups 2–7 were smaller than that of group 1, and there were significant decreases in the amount of spermine ($P < 0.01$). Significant decreases of total polyamine levels were found in groups 2, 6, and 7 ($P < 0.05$ or $P < 0.01$).

Enumeration of BrdUrd-labeled Cells. The results of morphometric analysis of BrdUrd labeling indices in the nonlesional squamous epithelium are summarized in Table 4. The mean BrdUrd labeling index number for the tongue epithelium exposed to 4-NQO alone (group 1) was the highest among the groups and was significantly larger than that of untreated control (group 11; $P < 0.01$; Table 5). Dietary administration of test chemicals (groups 2–7) significantly decreased those values when compared with group 1 ($P < 0.01$).

DISCUSSION

The results in the present study demonstrated that dietary administration of the flavonoids chalcone, 2-hydroxychalcone, and quercetin during the initiation or postinitiation phase effectively suppresses 4-NQO-induced oral carcinogenesis without any toxicity and pathological alteration of other organs in rats. 4-NQO is known to have two initial metabolic pathways. One pathway is the metabolic conversion of 4-NQO into 4-hydroxyaminoquinoline-1-oxide and 4-aminoquinoline 1-oxide, which is observed in prokaryotic and eukaryotic cells. It has been proposed that the mutagenic activity of 4-NQO is due to 4-hydroxyaminoquinoline-1-oxide, which binds covalently to nucleic acids and damages chromosomes through the formation of DNA adducts. The enzyme that catalyzes this reaction has been identified as NADPH:quinone oxidoreductase (DT-diaphorase). The other pathway is the detoxification of 4-NQO by its conversion to a glutathione conjugate (59–61). The balance between two pathways may be important in determining the carcinogenic potency of 4-NQO.

Quercetin has been reported to be mutagenic in the Ames test and other short-term *in vitro* tests (33–35), but its mutagenicity appears to be dependent on the absence of excision repair capability. In an *in vivo* test, Pamukcu *et al.* also reported that quercetin, administered in a special commercial chow to a specific strain of Norwegian rats, caused an increase in the incidence of intestinal and urinary bladder tumors (36). Moreover, Ertürk *et al.* reported that quercetin fed to female Sprague-Dawley and Fischer 344 rats was a hepatocarcinogen (37). However, a long-term, well-controlled feeding experiment with F344/DuCrj and ACI rats did not confirm the reported carcinogenicity of quercetin (62, 63). In several *in vitro* experiments, quercetin showed growth-inhibitory effects on cells from various human cancers, including tongue (38), ovarian (39), gastric (40), breast (41), colon (42), and leukemic (43) cells. Quercetin, however, had no significant effect on the growth of normal human embryonic lung fibroblasts except at the highest concentration tested (38). These results suggest that quercetin may have a selective inhibition of human tumor cell growth. Dietary administration of quercetin is known to inhibit mammary carcinogenesis (44) and colon tumorigenesis (45). Such an inhibitory effect of quercetin may be caused through several possible mechanisms. One possible mechanism to inhibit the development of chemically induced tumors might be the modulation and inhibition of the activity of enzymes that participate in metabolic activation of carcinogens. Quercetin is a potent inhibitor of epidermal cytochrome P-450-dependent microsomal metabolism (64). Elangovan *et al.* reported that quercetin inhibits lipid peroxidase and cytochrome P-450 and increases glutathione *S*-transferase (65). Quer-

Table 4 *Polyamine levels of tongue of rats in each group*

Group	Treatment	No. of rats examined	Polyamine levels (nmol/mg protein)			
			Diamine	Spermidine	Spermine	Total
1	4-NQO alone	15	0.08 ± 0.07[a]	1.56 ± 0.32[b]	1.84 ± 0.19[b]	3.48 ± 0.46[b]
2	4-NQO + chalcone (500 ppm)	16	0.05 ± 0.05	1.64 ± 0.36	1.49 ± 0.28[c]	3.17 ± 0.53[d]
3	4-NQO + 2-hydroxychalcone (500 ppm)	16	0.03 ± 0.06[d]	1.57 ± 0.44	1.51 ± 0.34[c]	3.12 ± 0.75
4	4-NQO + quercetin (500 ppm)	16	0.07 ± 0.11	1.52 ± 0.37	1.57 ± 0.28[c]	3.16 ± 0.58
5	4-NQO → chalcone (500 ppm)	16	0.02 ± 0.03[d]	1.67 ± 0.35	1.51 ± 0.23[c]	3.20 ± 0.49
6	4-NQO → 2-hydroxychalcone (500 ppm)	16	0.08 ± 0.10	1.53 ± 0.36	1.48 ± 0.21[c]	3.08 ± 0.48[d]
7	4-NQO → quercetin (500 ppm)	15	0.10 ± 0.18	1.44 ± 0.38	1.51 ± 0.19[c]	3.05 ± 0.41[c]
8	Chalcone (500 ppm)	8	0.08 ± 0.06	1.49 ± 0.34[e]	1.57 ± 0.26	3.14 ± 0.49
9	2-Hydroxychalcone (500 ppm)	8	0.06 ± 0.09	1.55 ± 0.36[e]	1.54 ± 0.28	3.15 ± 0.49
10	Quercetin (500 ppm)	6	0.09 ± 0.11	1.47 ± 0.18[e]	1.49 ± 0.15	3.05 ± 0.25
11	No treatment	8	0.09 ± 0.13	1.21 ± 0.26	1.59 ± 0.23	2.88 ± 0.36

[a] Mean ± SD.
[b] Significantly different from group 11 by Dunnett's test ($P < 0.01$).
[c] Significantly different from group 1 by Dunnett's test ($P < 0.01$).
[d] Significantly different from group 1 by Dunnett's test ($P < 0.05$).
[e] Significantly different from group 11 by Dunnett's test ($P < 0.05$).

Table 5 *BrdU labeling index of nonlesional area of tongue squamous epithelium*

Group	Treatment	No. of rats examined	BrdU labeling index (%)
1	4-NQO alone	15	7.75 ± 0.84[a,b]
2	4-NQO + chalcone (500 ppm)	16	6.50 ± 1.23[c]
3	4-NQO + 2-hydroxychalcone (500 ppm)	16	6.11 ± 0.64[c]
4	4-NQO + quercetin (500 ppm)	16	6.44 ± 0.94[c]
5	4-NQO → chalcone (500 ppm)	16	5.94 ± 0.96[c]
6	4-NQO → 2-hydroxychalcone (500 ppm)	16	6.18 ± 0.71[c]
7	4-NQO → quercetin (500 ppm)	15	6.32 ± 0.88[c]
8	Chalcone (500 ppm)	8	5.37 ± 1.02
9	2-Hydroxychalcone (500 ppm)	8	5.13 ± 0.63[d]
10	Quercetin (500 ppm)	6	5.19 ± 0.68
11	No treatment	8	6.10 ± 1.30

[a] Mean ± SD.
[b] Significantly different from group 11 by Dunnett's test ($P < 0.01$).
[c] Significantly different from group 1 by Dunnett's test ($P < 0.01$).
[d] Significantly different from group 11 by Dunnett's test ($P < 0.05$).

cetin also inhibits certain biochemical events associated with tumor promotion, such as alterations in protein kinase C (66). Moreover, quercetin is reported to arrest human leukemic T cells in the late G_1 phase of the cell cycle (43). These possible mechanisms may play some important roles for *in vivo* chemopreventive interactions. However, most of the ingested quercetin is either metabolized by the intestinal flora into phenolic acids or is excreted unchanged in the feces. Only traces of quercetin are absorbed from the gut (67).

Chalcones, including chalcone and 2-hydroxychalcone, are reported to be potent antimutagens (51). Also, chalcones have cytotoxic and antiproliferative effects on cells from various human cancers: gastric (49), pancreatic (49), neuroblastoma (49), cervical (49, 50), colon (52), and lymphoma ascites (48) cells. The mechanisms of such effects are not known, but possible mechanisms have been suggested. Compounds containing two aromatic rings joined by an α,β-unsaturated carbonyl system such as chalcone have been shown to bind to receptors that induce activities of phase II enzymes. Induction of increased activity of phase II enzymes has been shown to result in inhibition of initiation of chemical carcinogenesis (54). 2-Hydroxychalcone can affect the proliferation of HeLa cells by initially inhibiting DNA and RNA (50). Moreover, a derivative of chalcones, 3′-methyl-3-hydroxy-chalcone, causes G_0/G_1 arrest (49). In *in vivo* studies, dietary administration of chalcone has been reported to inhibit benzo(*a*)pyrene-induced pulmonary carcinogenesis in female A/J mice and *N*-methyl-*N*-nitrosourea-induced mammary carcinogenesis in female Sprague-Dawley rats (54, 55). The results in these studies indicate that chalcone exerts its chemopreventive activity as a suppressing agent, as does quercetin. In the present study, chalcone, 2-hydroxychalcone, and quercetin possess both blocking and suppressing properties in a 4-NQO-induced oral carcinogenesis model.

The mechanisms involved in the inhibition of the initiation or postinitiation events of oral carcinogenesis by quercetin and chalcones are not clear. However, it is supposed that these test chemicals have common biological characteristics. Quercetin and some derivatives of chalcone have been reported to have an antioxidative function and modulatory effects on lipoxygenase and cyclooxygenase pathways in the arachidonic acid cascade, which plays an important role in tumor promotion (46, 47, 68). The animal studies with the arachidonic acid cascade inhibitors as blocking and suppressing agents have been done with two nonsteroidal anti-inflammatory drugs, piroxicam and indomethacin (69). We have reported that these two synthetic compounds inhibit carcinogenesis of 4-NQO-induced rat oral carcinogenesis (21). One or more lipoxygenase products are involved in the induction of ornithine decarboxylase (47), the rate-limiting enzyme in the polyamine biosynthesis pathway that plays an essential role in cell proliferation and differentiation. In this study, we estimated cell proliferation using polyamine levels and the BrdUrd labeling index in the tongue epithelium. The results of both proliferation biomarkers indicate that the test chemicals have suppressing effects on cell proliferation in the target organ. Therefore, one of the mechanisms for chemopreventive activities of the test compounds may be related to suppression of cell proliferation, especially when fed the compounds during the postinitiation phase. Other mechanisms include the capacity to scavenge free radicals, to block or trap ultimate carcinogen electrophiles by forming innocuous products in a nucleophilic chemical reaction, or to enhance the activity of detoxification enzymes (70).

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