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SHORT COMMUNICATION

Inhibitory effect of dietary flavonol quercetin on 7,12-dimethylbenz[*a*]anthracene-induced hamster buccal pouch carcinogenesis

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The inhibitory effect of dietary supplementation with flavonol quercetin on 7,12-dimethylbenz[*a*]anthracene (DMBA)-induced hamster buccal pouch carcinogenesis was investigated. Dietary quercetin inhibited the incidence of both papillomas and tumors induced by DMBA. The fluorescence spectra of papillomas and tumors showed different prominent maxima and a characteristic peak around 620–630 nm, which could be attributed to the accumulation of porphyrin compounds. Further, the fluorescence intensities at 630 nm (FI_{630nm}) were elevated, whereas the ratio FI_{520nm}/FI_{630nm} was decreased in DMBA-induced lesions. Quercetin treatment significantly decreased FI_{630nm} and increased the ratio FI_{520nm}/FI_{630nm} when compared with DMBA-induced lesions. It is therefore evident that quercetin has an inhibitory effect on DMBA-induced carcinogenesis and further studies will throw more light on its use as a chemopreventive agent against oral cancer.

Bio-flavonoids constitute an integral part of the human diet. It has been estimated that the average dietary intake of flavonoids is ~1 g/day (1,2). Flavonoids are widespread among food plants, including vegetables and fruits, and the flavonol quercetin (3,3′,4′,5,7-pentahydroxyflavone) is found in the edible portion of a majority of dietary plants (e.g. citrus fruits, berries, leafy vegetables, roots, tubers and bulbs, herbs and spices, legumes, cereal grains, tea and cocoa) (1). Reports suggest that quercetin and certain related flavonoids may be inhibitors of the carcinogenic process (3–6). A number of hydroxylated flavonoids, including quercetin, have been found to inhibit the mutagenic activity of bay region diol epoxides of benzo[*a*]pyrene (7).

Recently we reported that light-induced fluorescence spectroscopy may be used to discriminate between pre-malignant and malignant tissues of 7,12-dimethylbenz-[*a*]anthracene (DMBA*)-induced hamster buccal pouch carcinogenesis and control tissues (8). Hence, the present study was designed to evaluate the effect of dietary quercetin on DMBA-induced oral carcinogenesis using light-induced fluorescence spectroscopy.

Male Syrian hamsters aged 4–6 weeks (National Institute of Nutrition, Hyderabad, India) were used in this study. The hamsters were fed *ad libitum* with commercial basal diet (Hindustan Lever Limited, Calcutta, India) and water. The flavonol quercetin (Bio-organics, Madras, India) was blended separately into the powdered pellet diet and stored in sealed containers at 4°C.

The animals were divided into four groups comprising 12 animals each. Group I animals received topical application of liquid paraffin using a brush on the right buccal mucosa three times per week for 16 weeks. Group II animals were fed a 2.0% quercetin diet. A 0.5% solution of the carcinogen DMBA (Sigma, St Louis, MO) in liquid paraffin was painted on the right buccal mucosa using a brush three times per week for 16 weeks for group III animals. Group IV animals received topical application of DMBA as group III and were fed the 2% quercetin diet as group II. Carcinogenesis induction by DMBA application was terminated at week 16 of experimentation.

The incidence of papillomatous outgrowths at week 10 and tumors (<2 mm in diameter) at week 16 of the experiment were counted. The animals were killed by cervical decapitation and the cheek pouches excised. The mucosal tissues were used for spectral studies as reported previously (8). The data were analysed statistically using Student's *t*-test.

Table I summarizes the effect of quercetin on the incidence of papillomas and tumors due to DMBA application. The control and quercetin-treated animals showed no papilloma or tumor incidence. The incidence of papilloma at week 10 and of tumor at week 16 in DMBA-induced animals was taken as 100%. A significant reduction in the mean papilloma ($P < 0.01$) and tumor incidence ($P < 0.001$) was observed in quercetin-treated animals.

Figure 1 shows the mean fluorescence spectra of buccal mucosa of control and experimental hamsters excited at 405 nm. The spectra of control and quercetin-treated control mucosa show maximum intensity at 430 nm and decrease towards longer wavelengths. The papilloma spectrum exhibited maximum intensity around 470 nm with a distinct peak around 628 nm, whereas in quercetin-treated papilloma the spectrum shows a maximum intensity at 430 nm and a decreased peak around 628 nm. The emission spectrum of DMBA-induced tumor shows a prominent peak around 628 nm, whereas the quercetin-treated tumor shows a maximum intensity at 485 nm and a significantly decreased peak around 628 nm.

The characteristics of the fluorescence spectra of buccal mucosa of control and experimental animals excited at 405 nm are presented in Table II. The fluorescent intensities (FI) at 630 nm and the ratio FI_{520nm}/FI_{630nm} were significantly elevated ($P < 0.001$) and decreased ($P < 0.001$) in papillomatous and tumor tissues respectively. Quercetin treatment significantly decreases the FI_{630nm} in both papilloma and tumor tissues when compared with untreated lesions. Furthermore, the ratio FI_{520nm}/FI_{630nm} was also increased significantly when compared with untreated controls.

Quercetin has been reported to reduce the incidence of DMBA-induced papillomas in mouse skin (3). The inhibitory effect of quercetin was further confirmed by the fluorescence spectra results obtained from quercetin-treated lesions.

*Abbreviations: DMBA, dimethylbenz[*a*]anthracene; FI, fluorescence intensity.

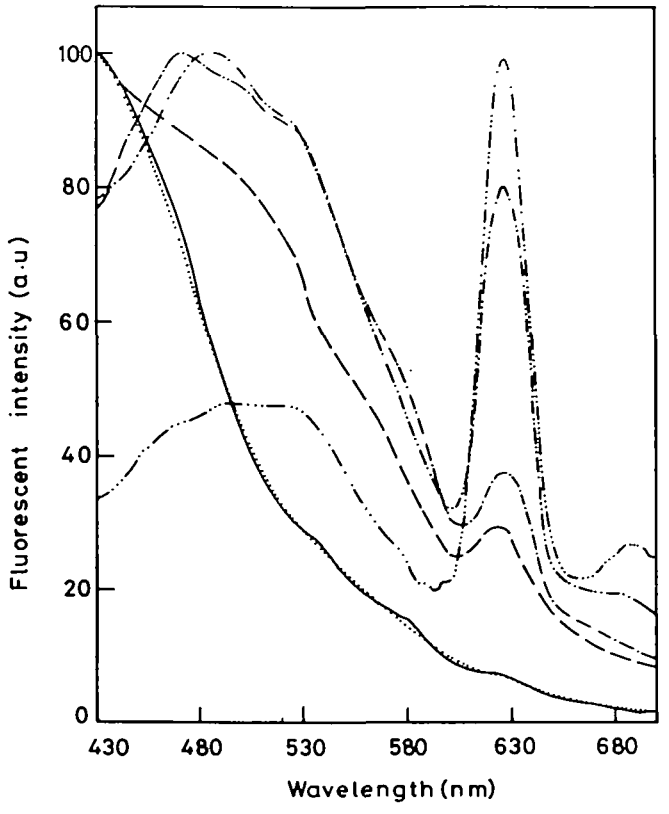

Fig. 1. Mean fluorescence spectra of buccal mucosa of control, quercetin-treated control, DMBA-induced lesions and quercetin-treated lesions at 405 nm excitation. ——— Control; ..... quercetin-treated control; —·—·— DMBA-induced papilloma; — — — quercetin-treated papilloma; —··— DMBA-induced tumor; —·— quercetin-treated tumor.

Table I. Incidence of papillomas and tumors in control and experimental hamsters

Group	Papillomas at week 10	Tumors at week 16
Control		
Quercetin-treated control		
DMBA-induced buccal lesions	3.4±0.60 (100%)	4.9±0.79 (100%)
Quercetin-treated buccal lesions	2.0±0.39[a] (58.8%)	3.1±0.47[a](63.2%)

The values are expressed as mean±SD for six animals in each group.
[a]$P < 0.001$. DMBA-induced buccal lesions versus quercetin-treated buccal lesions.

Quercetin treatment alters the maximum intensity, FI_{630nm} and the ratio FI_{520nm}/FI_{630nm} when compared with DMBA-induced lesions. The characteristic peak around 620–630 nm could be attributed to accumulation of porphyrin compounds in papilloma and tumor tissues, as reported previously (8). These results suggest that fluorescence spectroscopy may be used as a prognostic index for therapeutic studies, in addition to its diagnostic purpose.

From the present investigations it can be inferred that dietary quercetin showed significant inhibitory effect on DMBA-induced hamster buccal pouch carcinogenesis. The mechanisms involved in the alteration of light-induced fluorescence spectroscopy of DMBA-induced buccal lesions by quercetin treatment are unclear. However, several mechanisms may exist which may contribute to a decrease in DMBA-induced lesions. Quercetin has been shown to have free radical scavenging and antioxidant capacity (9,10). Recently Elangovan *et al.* (3) reported that quercetin enhanced the activity of glutathione S-transferase and glutathione levels and decreased the levels of lipid peroxides in DMBA-induced skin carcinogenesis in mice. Quercetin has been shown to inhibit the synthesis of DNA in fibrosarcoma cells (4). Recently we have reported decreased levels of antioxidants and elevated lipid peroxidation in pre-malignant and malignant tissues of DMBA-induced hamster buccal pouch carcinogenesis when compared with control tissues (11).

Table II. Characteristics of fluorescence spectra of buccal mucosa of control, quercetin-treated control, DMBA-induced lesions and quercetin-treated lesions at 405 nm excitation

Group	Prominent maxima (nm)	FI_{630nm}	FI_{520nm}/FI_{630nm}
Control	430±0.0	7.7±0.4	4.6±0.29
Quercetin-treated control	430±0.0	7.5±0.5	4.7±0.28
DMBA-induced papilloma	470±4.8	35.8±2.6[a]	2.5±0.15[a]
Quercetin-treated papilloma	430±0.0	29.5±2.1[a]	2.5±0.12
DMBA-induced tumor	628±0.0	96.9±1.9[a]	0.48±0.03[a]
Quercetin-treated tumor	485±3.4	81.2±4.6[a]	1.10±0.07[a]

The values are expressed as mean±SD for six animals in each group.
[a]$P < 0.001$. Control versus quercetin-treated control; control versus DMBA-induced papilloma; control versus DMBA-induced buccal carcinoma; DMBA-induced papilloma versus quercetin-treated papilloma and DMBA-induced buccal carcinoma versus quercetin-treated buccal carcinoma.

Quercetin has also been reported to inhibit epidermal cytochrome P450 monoxygenases (12) and DMBA–DNA adduct formation in SENCAR mice (13). The inhibitory effect of quercetin may also be due to its influence on the intercellular concentration of reactive metabolites of the carcinogen and/or carcinogen–DNA adduct formation.

The inhibitory effect of quercetin may be due to its various properties, such as free radical scavenging, antioxidant properties and inhibition of DNA synthesis, cytochrome P450 monoxygenases and DMBA–DNA adduct formation. Further research on quercetin may allow its adoption as a chemopreventive agent against oral cancer.

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