86. Cytogenetic Effects of Quercetin on Cultured Mammalian Cells^{*)}

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Quercetin (3,3',4',5,7-pentahydroxyflavone) has been known as one of ubiquitous flavonoid derivatives naturally occurring in a variety of plants used for food such as fruits, vegetables, tea and spices. Recently mutagenic activities of quercetin and some other flavonoids have received special attention in view of the possible cancer risk to man (Brown 1980). Quercetin has been reported to be a potent mutagen, with or without metabolic activation, in both bacterial (Bjerdanes and Chang 1977; Sugimura *et al.* 1977; Hardigree and Epler 1978; Brown and Dietrich 1979) and mammalian cell systems (Maruta *et al.* 1979). Its possible carcinogenic activity has also been revealed by the *in vitro* transformation assay with cryopreserved Syrian hamster embryo cells (Umezawa *et al.* 1977).

We have examined cytogenetic effects of quercetin as measured by the frequency of sister chromatid exchanges (SCEs) and chromosome aberrations.

Materials and methods. Quercetin (Tokyo Kasei Kogyo Co., Ltd.) was dissolved in dimethylsulfoxide (DMSO) and diluted with a culture medium, final concentration of DMSO being 0.02%.

A diploid human fibroblast cell line, HE2144, two pseudodiploid Chinese hamster fibroblastic cell lines, Don-6 and B-131, and PHA-stimulated human lymphocyte cultures from three healthy donors were used in the present experiments. Monolayer cells were grown in a modified Eagle's medium supplemented with 10–15% fetal calf serum (pH 7.2). Quercetin, with at least three tolerable doses, was added to cultures after three hours of seeding, together with 1.0 $\mu\text{g}/\text{ml}$ of 5-bromodeoxyuridine (BUdR). The cultures were kept in complete darkness at 37°C for two cell cycles; 26–30 hr in Don-6, 24–27 hr in B-131, 40–48 hr in HE2144, taking account of the mitotic delay caused by the chemical treatment. Human lymphocytes (HL) were

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cultured according to the routine phytohemagglutinin method with 0.5 ml of whole blood in 10 ml of the culture medium, to which quercetin and BUdR were added 24 hr after initiation of the culture, and the incubation was continued for further 48–51 hr. Chromosome slides were made according to the routine air-drying method following Colcemid (0.2 $\mu\text{g}/\text{ml}$ for final 2 hr) and hypotonic KCl (0.075 M) treatments and fixation with methanol-acetic acid (3:1). The frequency of SCEs was scored on 25 second round metaphase cells stained with the acridine orange fluorescence method (Kato 1974). Some slides were stained with Giemsa, and the frequency of chromosome aberrations was estimated on 100 metaphases.

Results and remarks. The results are presented in Table I. The

Table I. Frequencies of chromosome aberrations and sister chromatid exchanges in human and Chinese hamster cells exposed to quercetin

Cells	Dose ($\mu\text{g}/\text{ml}$)	CB/cell ^{*1}	SCE/cell $\pm$ S.E. (range)
HE2144	7.5	— ^{*2}	— ^{*2}
	5.0	0.06 ^{*3}	13.64 $\pm$ 0.92 (8–26) ^{*4}
	2.5	0.01	10.92 $\pm$ 0.69 (6–18) ^{*4}
	1.0	0.02	6.72 $\pm$ 0.47 (1–11) ^{*4}
	0	0.01	4.12 $\pm$ 0.47 (1–9)
HL	15.0	—	—
	12.5	0.04 ^{*3}	11.92 $\pm$ 0.86 (6–25) ^{*4}
	10.0	0.05 ^{*3}	11.24 $\pm$ 1.24 (5–37) ^{*4}
	8.0	0.02	9.16 $\pm$ 0.92 (3–17) ^{*4}
	0	0.01	4.4 $\pm$ 0.44 (1–10)
	12.5	0.15 ^{*3}	19.72 $\pm$ 1.70 (9–50) ^{*4}
	10.0	0.03	10.96 $\pm$ 0.77 (5–21)
	8.0	0.05 ^{*3}	9.92 $\pm$ 0.51 (5–15)
	0	0.02	8.12 $\pm$ 0.69 (2–15)
	10.0	0.02	10.88 $\pm$ 0.81 (4–20) ^{*4}
	8.0	0.02	10.52 $\pm$ 0.95 (3–23)
	5.0	0.02	7.48 $\pm$ 0.55 (3–16)
	0	0.00	7.76 $\pm$ 0.53 (1–14)
Don-6	15.0	0.86 ^{*3}	26.84 $\pm$ 2.68 (9–64) ^{*4}
	12.5	0.67 ^{*3}	22.72 $\pm$ 1.49 (11–44) ^{*4}
	10.0	0.74 ^{*3}	17.24 $\pm$ 1.15 (6–31) ^{*4}
	0	0.11	10.08 $\pm$ 0.66 (5–18)
B-131	7.5	— ^{*2}	— ^{*2}
	5.0	0.27 ^{*3}	12.48 $\pm$ 0.82 (5–20) ^{*4}
	1.0	0.12 ^{*3}	7.72 $\pm$ 0.69 (3–16)
	0	0.01	6.40 $\pm$ 0.49 (0–11)

^{*1} Chromatid breaks per cell, excluding gaps. ^{*2} The absence of mitotic cells. ^{*3} Values higher than the highest control level.

^{*4} Statistically significant increase ($p < 0.01$).

frequency of SCEs after exposure to various doses of quercetin increased to 1.7–3.3 times the control values, depending on the type of cells used (Fig. 1, Table I). The increment of SCEs in the present

Fig. 1. Metaphase chromosomes of human lymphocyte exposed to quercetin (12.5 $\mu\text{g}/\text{ml}$), showing 19 SCEs (arrow heads).

Fig. 2. Quercetin-induced chromosome aberrations of human lymphocyte. a, acentric fragment. b, chromatid break.

four cell systems was statistically significant ($p < 0.01$), with an apparently dose-dependent response. The minimum doses with respect to the statistically significant increase of SCEs were 1.0 $\mu\text{g}/\text{ml}$ in HE2144, 5.0 $\mu\text{g}/\text{ml}$ in B-131, 8.0 $\mu\text{g}/\text{ml}$ in HL and 10.0 $\mu\text{g}/\text{ml}$ in Don-6, within the dose ranges employed. It was hence assumed that HE2144 was the most sensitive cell system in terms of the induction of SCEs by quercetin. On the other hand, the maximal tolerable doses, without

mitotic inhibition, were 5.0 $\mu\text{g/ml}$ for HE2144 and B-131, 10.0–12.5 $\mu\text{g/ml}$ for HL and 15.0 $\mu\text{g/ml}$ for Don-6.

The chromosome aberrations observed were mostly represented by chromatid breaks or gaps, iso-chromatid breaks, acentric fragments, and chromatid exchanges (Fig. 2). Although dicentrics and rings were occasionally found in Don-6 cells, with highly variable frequencies (0–10%) in both control and quercetin-treated cultures, they were excluded from counts. The highest frequencies of chromosome aberrations in control cultures so far obtained in the present study as well as in our previous series of similar experiments were 0.04 for HE2144, 0.02 for HL, 0.11 for Don-6 and 0.05 for B-131. In contrast, the aberration frequencies in quercetin-treated cultures were elevated up to 1.5–7.8 times the highest control level, although no clear-cut dosage effect was shown in HE2144 and HL. Furthermore, a considerable extent of individual difference was noted in the frequency of aberrations in HL. The reason for this remains to be solved.

In conclusion, the results of the present experiments indicate that quercetin induces chromosome breakages and SCEs in both human and Chinese hamster cells in culture, without exogenous metabolic activation. It should be mentioned here that edible bracken has long been known to contain certain carcinogenic principles (Evans and Mason 1965; Pamukcu and Price 1979; Hirono *et al.* 1970), and further that kaempferol, a structural analogue of quercetin isolated from bracken, showed significantly high mutagenic activities in *Salmonella* and mammalian cell systems (Sugimura *et al.* 1977; Brown and Dietrich 1979; Maruta *et al.* 1979). In view of the above features as well as the ubiquitous distribution of quercetin, extensive studies on its *in vivo* carcinogenic activity have been going on in several laboratories (Saito *et al.* 1980; Pamukcu *et al.* 1980).

Summary. Quercetin, a ubiquitous flavonoid, was found to induce chromosome aberrations and sister chromatid exchanges (SCEs) in cultured human and Chinese hamster cells without the application of metabolic activation systems. The increment in the frequency of SCEs was dose dependent, the maximum rates being 1.7–3.3 times the control values. The frequency of chromosome aberrations was elevated up to 1.5–7.8 times the highest control level, although clear dosage effects were obtained only in Chinese hamster cells.

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