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Quercetin, the active phenolic component in kiwifruit, prevents hydrogen peroxide-induced inhibition of gap-junction intercellular communication

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We evaluated the effects of the two main kiwifruit cultivars (gold kiwifruit (GOK) and green kiwifruit (GRK)) and their active phenolic compound, quercetin, on H_2O_2-induced inhibition of gap-junction intercellular communication (GJIC) in WB-F344 rat liver epithelial cells. We found that both GOK and GRK protect WB-F344 cells from H_2O_2-induced inhibition of GJIC. The extracellular signal-regulated protein kinase 1/2 (ERK1/2)–connexin 43 (Cx43) signalling pathway is crucial for the regulation of GJIC, and both GOK and GRK blocked the H_2O_2-induced phosphorylation of Cx43 and ERK1/2 in WB-F344 cells. Quercetin alone attenuated the H_2O_2-mediated ERK1/2–Cx43 signalling pathway and consequently reversed H_2O_2-mediated inhibition of GJIC in WB-F344 cells. A free radical-scavenging assay using 1,1-diphenyl-2-picrylhydrazyl showed that the scavenging activity of quercetin was higher than that of a synthetic antioxidant, butylated hydroxytoluene, per mol, suggesting that the chemopreventive effect of quercetin on H_2O_2-mediated inhibition of ERK1/2–Cx43 signalling and GJIC may be mediated through its free radical-scavenging activity. Since the carcinogenicity of reactive oxygen species such as H_2O_2 is attributable to the inhibition of GJIC, GOK, GRK and quercetin may have chemopreventive potential by preventing the inhibition of GJIC.

Connexin 43: Extracellular signal-regulated protein kinase 1/2: Gap-junction intercellular communication: Kiwifruit: Quercetin

Gap-junction intercellular communication (GJIC) is an important mode of cell–cell communication to help maintain homeostasis by facilitating direct exchanges of essential cellular metabolites and messengers less than 1–2 kDa including Na, K, Ca, cyclic AMP and ATP in multicellular organisms[1,2]. Multiple lines of evidence indicate that GJIC is dysregulated in most cancer cells and that its inhibition is strongly related to carcinogenesis[3,4]. Most tumour promoters, such as pesticides, peroxisome proliferators and dietary additives, are reported to inhibit GJIC; however, anti-tumour drugs can reverse GJIC disruption[5–7]. GJIC is regulated by gap-junction proteins connexins, and the closure of gap junctions is particularly mediated by phosphorylation-modulated conformational changes of connexin 43 (Cx43)[8].

Epidemiological studies indicate that a diet rich in antioxidant-containing fruits and vegetables can reduce the risk of cancer[9–11]. Specifically, kiwifruits have been used for the treatment of lung, liver and gastrointestinal (primarily stomach) cancers in traditional Chinese medicine, due to high levels of antioxidants[12–14]. Kiwifruit provides protection against oxidative DNA damage and enhances DNA repair, thereby protecting against mutagenic changes that can lead to transformation[15]. Kiwifruit inhibited sarcoma 180 growth in mice by 30–40 %[13]. Further, kiwifruit juice inhibited the growth of cancer cells *in vitro* [16,17], and some kiwifruit extracts showed a high 95 % inhibition of cancer growth by Ames' test[13].

Expressed on a fresh-weight basis, kiwifruit possesses a total phenol content of 274 mg/100 g[18]. Although analyses of total phenolics in kiwifruit indicated the level of phenolic compounds to be low in comparison with other fruits[19], a previous study showed that quercetin (3,3′,4′,5,7-pentahydroxyflavone) is found in kiwifruit juice as the glycosides of quercetin[20], and that quercetin is an important phenolic antioxidant in kiwifruit[21]. Depending on the methods of preparation, kiwi extract contains 0·63–1·06 mg quercetin glycosides/l[20]. Epidemiological studies have shown that quercetin consumption reduces the risk of developing cancer[22]. Further, the chemopreventive activity of quercetin has been demonstrated in a variety of laboratory animal

Abbreviations: BHT, butylated hydroxytoluene; Cx43, connexin 43; DPPH, 1,1-diphenyl-2-picrylhydrazyl; ERK1/2, extracellular signal-regulated protein kinase 1/2; GJIC, gap-junction intercellular communication; GOK, gold kiwifruit; GRK, green kiwifruit.

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models, including azoxymethane-induced colonic tumorigenesis in mice[23]. Additionally, quercetin is reported to prevent the appearance of pre-neoplastic lesions in rat hepatocarcinogenesis[24].

Although the potent chemopreventive activity of kiwifruit and quercetin has been demonstrated repeatedly, the molecular mechanisms underlying these effects are largely unknown. Recent reports suggest that the carcinogenicity of oxidative stress is attributable to the inhibition of GJIC[2]. Particularly, H_2O_2 is a well-known cancer promoter that disrupts GJIC[25–27]. H_2O_2 inhibits GJIC in WB-F344 rat liver epithelial cells with a 50 % inhibition (I_{50}) value of 200 μM[27]. WB-F344 cells are stimulated directly by H_2O_2 and H_2O_2 promotes proliferation and transformation of WB-F344 cells[28]. H_2O_2-mediated interference of GJIC has been reported to particularly correlate with the phosphorylation of Cx43 and extracellular signal-regulated protein kinase (ERK)[27,29]. The present study was designed to investigate the effects of both the gold kiwifruit (GOK) and green kiwifruit (GRK) cultivars, and their active phenolic phytochemical quercetin, on the H_2O_2-mediated inhibition of GJIC.

Materials and methods

Reagents

1,1-Diphenyl-2-picrylhydrazyl (DPPH), lucifer yellow, SDS, acrylamide, H_2O_2, quercetin and butylated hydroxytoluene (BHT) were obtained from Sigma (St Louis, MO, USA). The antibodies against phosphorylated ERK1/2 (Tyr202/Tyr204) and total ERK1/2 were purchased from Cell Signaling Technology (Beverly, MA, USA). The antibody against Cx43 was purchased from Zymed (San Francisco, CA, USA). All other chemicals were obtained from Fisher (Springfield, NJ, USA).

Preparation of kiwifruit extracts

The two main kiwifruit cultivars, GOK, which has a yellow colour and non-astringent taste, and GRK, which has a green colour and astringent taste, were supplied by the New Zealand Kiwifruit Marketing Board (ZESPRI). These kiwifruits were carefully pared, frozen and dried. The freeze-dried kiwifruits were ground to powder and stored at −20°C until used. Kiwifruit extracts were generated by mixing 10 g lyophilised kiwifruit with 100 ml of 80 % aqueous methanol. The kiwifruit–methanol mixture was sonicated for 20 min with continuous N_2 gas purging. The mixture was filtered through Whatman no. 2 filter paper (Whatman International Ltd, Maidstone, Kent, UK) using a chilled Buchner funnel and then rinsed with 50 ml methanol. The solid filter cake was then re-extracted by repeating the above steps under the same conditions. The two filtrates were combined, and an additional 50 ml of 80 % aqueous methanol was added. The solvent was evaporated using a rotary evaporator under reduced pressure at 40°C. The extract was dissolved in 50 ml of 100 % methanol and made up to the final volume of 100 ml with distilled deionised water. The solution was then centrifuged in a Sorvall® RC-5B Refrigerated Superspeed Centrifuge (Du Pont, Wilmington, DE, USA) at 12 000 ***g*** for 20 min. The final extracted product was stored at −4°C until used.

Cell culture

WB-F344 rat liver epithelial cells (WB-F344 cells) were kindly provided by Dr J. E. Trosko (Michigan State University, MI, USA). WB-F344 cells were cultured in Dulbecco's modified Eagle's medium (GIBCO, Gaithersburg, MD, USA), supplemented with 10 % fetal bovine serum (GIBCO) and penicillin–streptomycin (GIBCO) at 37°C in a 5 % CO_2 humidified incubator (Forma Scientific, Marietta, OH, USA).

Bioassay of gap-junction intercellular communication

GJIC was measured by the scrape-loading–dye-transfer technique as described previously[26]. Briefly, WB-F344 cells were pre-incubated with kiwifruit extract or quercetin for 30 min and then stimulated with 100 μM-H_2O_2 for 1 h. After H_2O_2 treatment, cells were washed twice with PBS. Next, lucifer yellow was added to the washed cells, and three scrapes were made using a scalpel with a surgical-steel blade under low light intensity. Each scrape traversed a large group of confluent cells. After 3 min of incubation, the cells were washed four times with PBS and then fixed with a 4 % formalin solution. Communicating cells showing green fluorescence were distinguishable from cells that are not communicating under an inverted fluorescence microscope (I × 70; Olympus, Okaya, Japan). The number of communicating cells was counted.

Western blot analysis

Western blot analysis was performed to measure the protein level of Cx43, ERK1/2 and phosphorylated ERK1/2. Briefly, total cell lysates were suspended in 4 × sample buffer (8 % SDS, 20 % glycerin, 250 mM-2-amino-2-(hydroxymethyl) propane-1,3-diol (Tris)-HCl (pH 7·5), 0·2 % bromophenol blue and 40 mM-dithiothreitol), heated at 95°C for 5 min and separated by SDS–PAGE on a 12·5 % polyacrylamide gel. The proteins were then transferred to a 0·45 μm polyvinylidene fluoride transfer membrane (Gelman Laboratories, Ann Arbor, MI, USA) and incubated in a blocking buffer (25 mM-Tris-HCl (pH 7·5), 150 mM-NaCl, 0·05 % Tween-20 and 5 % skimmed milk). Blots were probed with primary antibodies and then horseradish peroxidase-conjugated goat anti-mouse IgG secondary antibodies (Zymed Laboratories), according to the manufacturer's instructions. For visual detection, blots were developed using an enhanced chemiluminescence system (Amersham Biosciences, Little Chalfont, Bucks, UK).

1,1-Diphenyl-2-picrylhydrazyl radical-scavenging activity assay

The DPPH radical-scavenging activities of resveratrol and BHT were measured using the method described by Brand-Williams *et al.* with minor modifications[30,31]. DPPH was dissolved in 80 % aqueous methanol. A 0·1 ml volume of quercetin or BHT at various concentrations was added to 2·9 ml of the DPPH radical solution. The mixture was then shaken vigorously and incubated in the dark at 23°C for 30 min. Absorbance at 517 nm was measured using a spectrophotometer (Hitachi, Tokyo, Japan).

Statistical analysis

All experiments were repeated at least three times unless otherwise stated. Results are presented as mean values and standard deviations of triplicates. Comparisons between two groups were analysed using Student's *t* test. Probability values of $P<0{\cdot}05$ were considered statistically significant.

Results

Kiwifruit extracts prevented hydrogen peroxide-induced inhibition of gap-junction intercellular communication

The effects of two kiwifruit extracts on the H_2O_2-mediated inhibition of GJIC in WB-F344 cells were assessed using the scrape-loading–dye-transfer technique. The distance that the dye travelled perpendicular to the scrape was observed under an inverted fluorescence microscope as shown in Fig. 1(a). Untreated WB-F344 cells exhibited active GJIC (Fig. 1(a–i)). Treatment of cells with 100 μM-H_2O_2 for 1 h resulted in the dye travelling a shorter distance from the scrape, indicating an inhibition of GJIC (Fig. 1(a-ii)). WB-F344 cells pre-treated with 10 and 20 mg/ml GOK and GRK extracts for 30 min showed a reduction in the H_2O_2-induced inhibition of GJIC.

The data were quantified by normalising the number of communicating WB-F344 cells in the untreated control to 100 % (Fig. 1(b-i)). Treatment of cells with 100 μM-H_2O_2 for 1 h reduced GJIC to about 35 % (Fig. 1(b-ii)). However, pre-treatment of cells with 20 mg/ml of either GOK or GRK extracts completely prevented the H_2O_2-induced inhibition of GJIC (Fig. 1(b-iv and vi)).

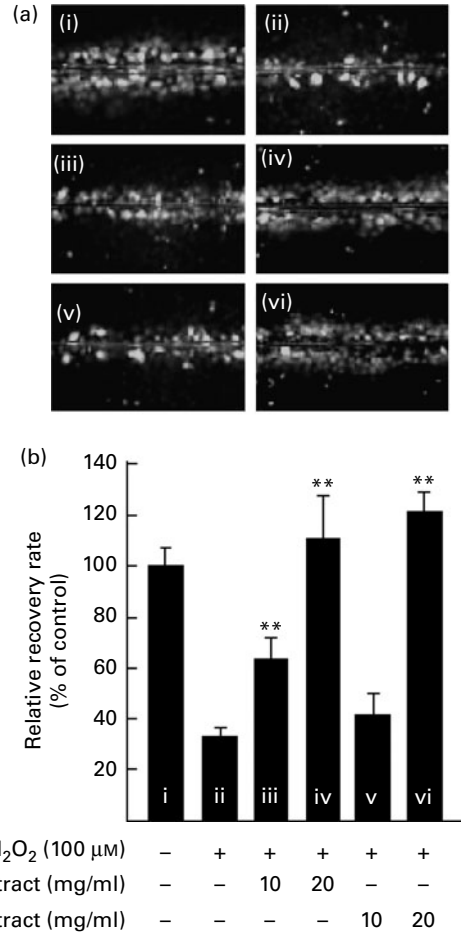

Fig. 1. Effects of gold kiwifruit (GOK) and green kiwifruit (GRK) extracts on the H_2O_2-induced inhibition of gap-junction intercellular communication (GJIC) in WB-F344 cells. GJIC was assessed using the scrape-loading–dye-transfer method under an inverted fluorescence microscope (100 ×). (a) Representative photographs of the following treatments: (i) untreated control (distilled deionised water (ddH_2O) as vehicle) for 30 min; (ii) H_2O_2 (100 μM) for 1 h; (iii) GOK extract (10 mg/ml) for 30 min and then H_2O_2 (100 μM) for 1 h; (iv) GOK extract (20 mg/ml) for 30 min and then H_2O_2 (100 μM) for 1 h; (v) GRK extract (10 mg/ml) for 30 min and then H_2O_2 (100 μM) for 1 h; (vi) GRK extract (20 mg/ml) for 30 min and then H_2O_2 (100 μM) for 1 h. (b) The number of communicating cells was counted, and the number of communicating cells in the untreated control was normalised to 100 %. The relative rate of GJIC for each of the treatment conditions was calculated as the percentage of the untreated control. Values are means (*n* 3), with standard deviations represented by vertical bars. ** The mean number of communicating cells was significantly higher than that in the H_2O_2-only treatment ($P<0{\cdot}01$).

Kiwifruit extracts blocked hydrogen peroxide-induced phosphorylation of connexin 43 and extracellular signal-regulated protein kinase 1/2

H_2O_2-mediated inhibition of GJIC has been reported to correlate with the phosphorylation of Cx43 and ERK[27,29]. To determine if kiwifruit extracts function by blocking the phosphorylation of these proteins, Western blot analysis with antibodies specific to Cx43, phosphorylated ERK1/2 and total ERK1/2 was performed (Fig. 2). Four bands corresponding to Cx43 (P0, P1, P2 and P3) were detected in the untreated control WB-F344 cells (Fig. 2(a)). The electrophoretic mobility of Cx43 is slower (upward shifted) according to the degree of phosphorylation. The P0 band represents non-phosphorylated Cx43, P1 is mono-phosphorylated, and the P2 and P3 bands represent hyper-phosphorylated Cx43. The ratios of phosphorylated Cx43 were demonstrated in densitometry scans for the quantitative analysis. The horizontal lines represent the position of each phosphorylated Cx43. In a vertical lane, the more a band is intensive, the more the graph moves from left to right. In the present study, data are representative of three independent experiments. Treatment of WB-F344 cells with 20 mg/ml of either GOK or GRK for 30 min had no effect on the phosphorylation status of Cx43. Treatment of WB-F344 cells with H_2O_2 at 100 μM for 1 h caused a decrease in the P0 and P1 bands and a concomitant increase in the P2 and P3 bands, indicating that H_2O_2 activated the hyper-phosphorylation of Cx43 (Fig. 2(a)). Pre-treatment with 20 mg/ml of GOK or GRK extracts for 30 min blocked the H_2O_2-mediated hyper-phosphorylation of Cx43, measured as a decrease in the (P2 + P3):(P0 + P1) ratio.

To identify the molecular mechanism by which kiwifruit extracts prevent H_2O_2-mediated inhibition of GJIC, we examined the phosphorylation status of ERK1/2 (Fig. 2(b)). Treatment of WB-F344 cells with 20 mg/ml of either GOK or GRK for 30 min had no effect on the phosphorylation of ERK1/2. In contrast, treatment with 100 μM-H_2O_2 for 1 h induced ERK1/2 phosphorylation. However, pre-treatment of WB-F344 cells with 20 mg/ml of GOK and GRK for 30 min blocked H_2O_2-induced phosphorylation of ERK1/2.

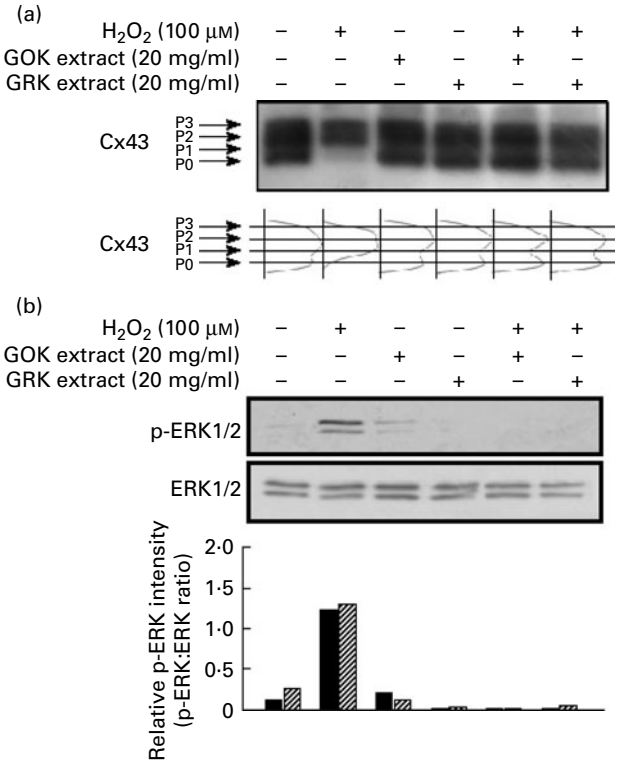

Fig. 2. Effects of gold kiwifruit (GOK) and green kiwifruit (GRK) extracts on the H_2O_2-induced phosphorylation of connexin 43 (Cx43) and extracellular signal-regulated protein kinase 1/2 (ERK1/2) in WB-F344 cells: lane 1, untreated control (distilled deionised water (ddH_2O) as vehicle for 1 h); lane 2, H_2O_2 (100 μM) for 1 h; lane 3, GOK extract (20 mg/ml) for 30 min; lane 4, GRK extract (20 mg/ml) for 30 min; lane 5, GOK extract (20 mg/ml) for 30 min and then H_2O_2 (100 μM) for 1 h; lane 6, GRK extract (20 mg/ml) for 30 min and then H_2O_2 (100 μM) for 1 h. (a) The phosphorylation status of Cx43 was analysed by Western blot analysis as described in the Materials and methods. The diagram shows the intensity of each Cx43 band. (b) The levels of phosphorylated ERK1/2 (p-ERK1/2) and total ERK1/2 were analysed by Western blot analysis as described in the Materials and methods. The bar graphs (bottom) show the p-ERK:ERK ratio, which was determined using a densitometry image analysis program. (■), p-ERK-1:ERK-1; (▨), p-ERK-2:ERK-2.

Quercetin prevented hydrogen peroxide-induced inhibition of gap-junction intercellular communication

Previous studies have shown that quercetin and quercetin glycosides are the active phenolic compounds found in kiwifruit[20]. Since kiwifruit extracts protected against H_2O_2-induced inhibition of GJIC, we next sought to determine if purified quercetin prevented H_2O_2-mediated inhibition of GJIC in WB-344 cells. Untreated WB-F344 cells had active GJIC (Fig. 3(a-i)); however, treatment with 100 μM-H_2O_2 for 1 h resulted in a clear inhibition of GJIC (Fig. 3(a-ii)). In contrast, pre-treatment of WB-F344 cells with 25 or 100 μM-quercetin for 30 min before H_2O_2 exposure reduced the extent of H_2O_2-mediated inhibition of GJIC. As above, the data were quantified by normalising the number of communicating WB-F344 cells in the untreated control to 100 % (Fig. 3(b-i)). Treatment of WB-F344 cells with 100 μM-H_2O_2 for 1 h decreased the GJIC by about 50 % (Fig. 3(b-ii)). Pre-treatment with 25 or 100 μM-quercetin completely blocked the H_2O_2-induced inhibition of GJIC (Fig. 3(b-iii and iv)).

Quercetin blocked hydrogen peroxide-induced phosphorylation of connexin 43 and extracellular signal-regulated protein kinase 1/2

We next sought to determine if quercetin was the component of kiwifruit extracts responsible for blocking the H_2O_2-induced phosphorylation of Cx43 and ERK1/2 and thereby preventing the inhibition of GJIC. As shown above, treatment of WB-F344 cells with 100 μM-H_2O_2 for 1 h induced the hyper-phosphorylation of Cx43 (Fig. 4(a)) and phosphorylation of ERK1/2 (Fig. 4(b)). Pre-treatment of WB-F334 cells with 25 or 100 μM-quercetin for 30 min decreased the ratio of hyper-phosphorylated to non- or mono-phosphorylated Cx43 ((P2 + P3):(P0 + P1)) induced by H_2O_2 treatment (Fig. 4(a)). Further, quercetin pre-treatment also blocked the H_2O_2-induced phosphorylation of ERK1/2 (Fig. 4(b)).

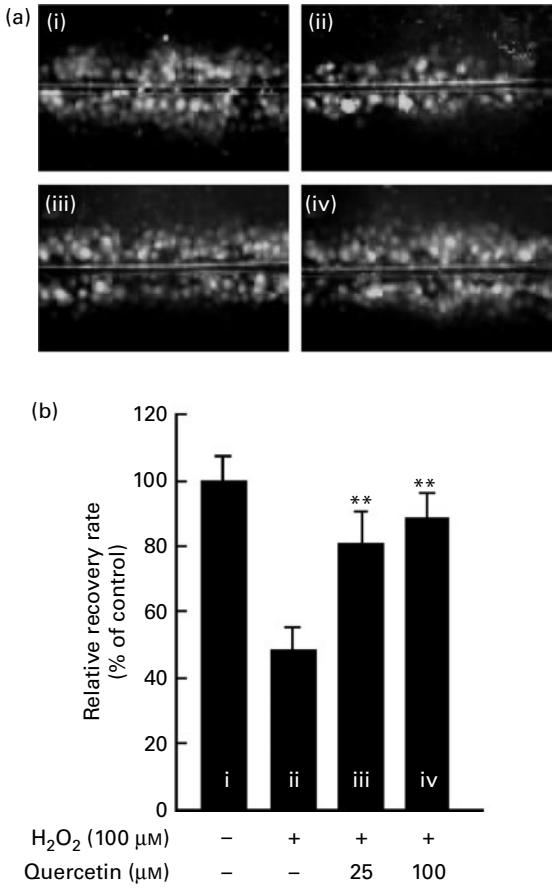

Fig. 3. Effects of quercetin on the H_2O_2-induced inhibition of gap-junction intercellular communication (GJIC) in WB-F344 cells. (a) GJIC was assessed using the scrape loading–dye transfer method under an inverted fluorescence microscope (100 ×). Representative photographs of each treatment: (i) Untreated control (distilled deionised water (ddH_2O) as vehicle for 1 h); (ii) H_2O_2 (100 μM) for 1 h; (iii) quercetin (25 μM) for 30 min and then H_2O_2 (100 μM) for 1 h; (iv) quercetin (100 μM) for 30 min and then H_2O_2 (100 μM) for 1 h. (b) The number of communicating cells was counted and the number of communicating cells in the untreated control was normalised to 100 %. The relative rate of GJIC for each of the treatment conditions was calculated as the percentage of the untreated control. Values are means (n 3), with standard deviations represented by vertical bars. ** The mean number of communicating cells was significantly higher than that in the H_2O_2-only treatment ($P<0·01$).

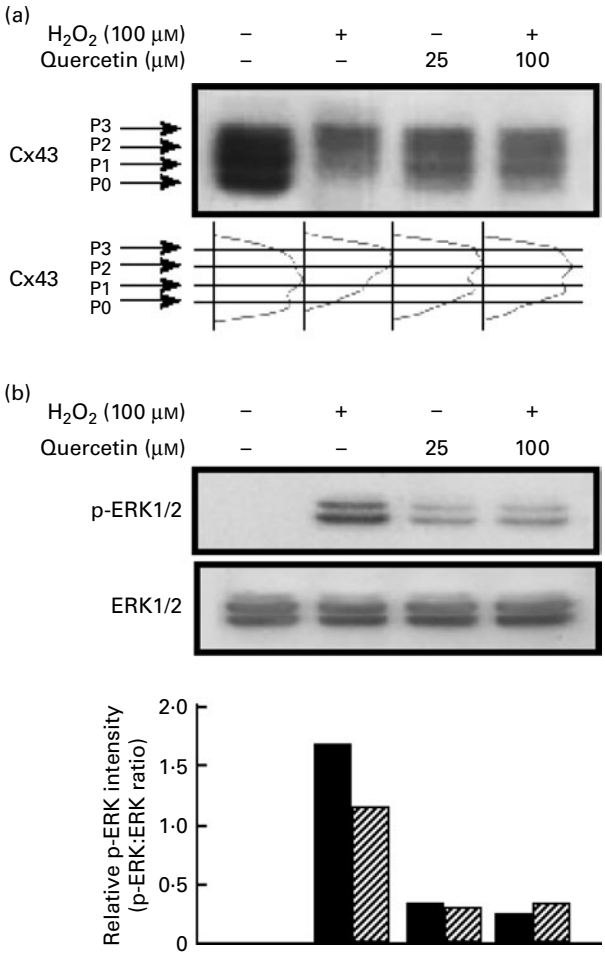

Fig. 4. Effects of quercetin on the H_2O_2-induced phosphorylation of connexin 43 (Cx43) and extracellular signal-regulated protein kinase 1/2 (ERK1/2) in WB-F344 cells: lane 1, untreated control (distilled deionised water (ddH_2O) as vehicle for 1 h); lane 2, H_2O_2 (100 μM) for 1 h; lane 3, quercetin (25 μM) for 30 min and then H_2O_2 (100 μM) for 1 h; lane 4, quercetin (100 μM) for 30 min and then H_2O_2 (100 μM) for 1 h. (a) The phosphorylation status of Cx43 was analysed by Western blot analysis as described in the Materials and methods. The diagram shows the intensity of each Cx43 band. (b) The levels of phosphorylated ERK1/2 (p-ERK1/2) and total ERK1/2 were analysed by Western blot analysis as described in the Materials and methods. The bar graphs (bottom) show the p-ERK:ERK ratio, which was determined using a densitometry image analysis program. (■), p-ERK-1:ERK-1; (▨), p-ERK-2:ERK-2.

Free radical-scavenging activity of quercetin

We measured the free radical-scavenging activity of quercetin and compared its activity with BHT, a synthetic phenolic antioxidant used in foods. The amount of free radicals produced by DPPH alone was normalised to 100 %, and increasing amounts of BHT or quercetin were added to assess their activities (Fig. 5). Both BHT and quercetin exhibited dose-dependent free radical-scavenging activity. Importantly, 50 or 100 μM-quercetin efficiently scavenged the free radicals produced by DPPH and had superior scavenging activity to that of BHT. As shown above, pre-treatment of cells with these concentrations of quercetin suppressed the phosphorylation of Cx43 and ERK1/2, thereby preventing the inhibition of GJIC and suggesting a possible link between the free radical-scavenging activity and protection of GJIC.

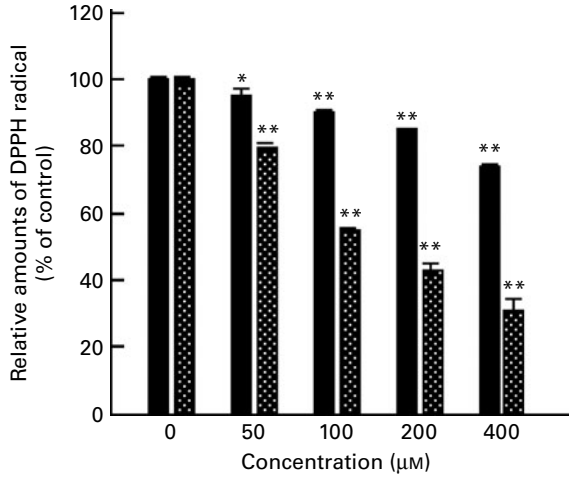

Fig. 5. Free radical-scavenging activity of butylated hydroxytoluene (BHT; ■) and quercetin (▩). The relative amount of free radicals generated by 1,1-diphenyl-2-picrylhydrazyl (DPPH) was determined as described in the Materials and methods. The amount of free radicals generated by DPPH was set at 100 % and the relative amount of each free radical after treatment with BHT and quercetin was evaluated. Values are means (n 3), with standard deviations represented by vertical bars. Mean value was significantly lower than than of the untreated control: * $P<0{\cdot}05$, ** $P<0{\cdot}01$.

Discussion

In the present report, we used a cell culture-based model system to evaluate the ability of kiwifruit extracts or purified quercetin, the active phenolic compound found in kiwifruit, to prevent H_2O_2-induced inhibition of GJIC. WB-F344 cells that were pre-treated with an extract from either the GOK or GRK cultivar maintained normal GJIC after challenge with 100 μM-H_2O_2 (Fig. 1). Further, GOK- or GRK-treated cells had a reduced level of Cx43 and ERK1/2 phosphorylation upon H_2O_2 treatment, and phosphorylation of these proteins is known to be associated with impaired GJIC. The protective effects of the kiwifruit extracts were mimicked by the treatment of cells with quercetin alone, indicating that quercetin is the active component of the kiwifruit extracts. Quercetin also had substantial free radical-scavenging activity, which was significantly greater than the synthetic phenolic antioxidant BHT. Together, these data suggest that the protective effects of quercetin on GJIC may be mediated by its antioxidant properties.

Many population-based studies highlight the association between diets rich in the macronutrients and micronutrients found in fruits and vegetables with a reduced risk of developing cancer[32]. Kiwifruits have been shown to have cancer-preventative activities in several cancer models[13,15–17]; however, the active compounds in kiwifruits that exert these anti-tumour activities have not been clearly identified. Recently, attention has been focused on phytochemicals, which are non-nutritive components of plant-based diets that possess cancer-preventive properties[32]. These phytochemicals act to prevent cancer by blocking the initiation or reversing the promotion of carcinogenesis[32]. Quercetin is an antioxidant found in substantial quantities in kiwifruits[20,21], suggesting that quercetin may be the primary phytochemical in kiwifruits that is responsible for the cancer-preventative properties.

Multiple lines of evidence indicated that the inhibition of GJIC is a carcinogenic and tumour-promoting process[3,4]. Most normal cells exhibit functional GJIC, but most cancer cells exhibit impaired GJIC[3,4]. Natural chemopreventive compounds, such as germanium dioxide[5], honeybee propolis[33], green tea and components of Korean ginseng[7,29] have been shown to prevent or abolish inhibition of GJIC. Here we found that kiwifruit extracts from both GOK and GRK, as well as quercetin, blocked H_2O_2-induced inhibition of GJIC, suggesting that the chemopreventive activity of kiwifruits and quercetin may be mediated by preventing the inhibition of GJIC from oxidative stress-mediated injury.

The mechanism by which oxidative stress inhibits GJIC involves conformational changes in gap junctions due to the phosphorylation of Cx43, a major component of gap-junction channels[5,33,34]. Inhibition of GJIC also involves the activation of mitogen-activated protein kinases (MAPK)[26]. In particular, phosphorylation of ERK was reported to play a key role in the inhibition of GJIC *in vitro*[35,36]. Pharmacological inhibitor of MAPK kinase 1/2 (MEK1/2, an ERK upstream kinase) recovered H_2O_2-induced inhibition of GJIC up to 100 %; however, an inhibitor of p38 MAPK showed only partial protection[37]. A pharmacological inhibitor of c-Jun N-terminal kinases (JNK) did not show any protection (data not shown). Previous studies demonstrated that phosphorylation of Cx43 and ERK1/2 are important events controlling H_2O_2-induced inhibition of GJIC in WB-F344 cells[25,26]. We observed that kiwifruit extracts from both GOK and GRK, and quercetin alone, blocked H_2O_2-induced phosphorylation of ERK1/2 and Cx43, thereby protecting GJIC in WB-F344 cells. These data suggest that quercetin mediates its effects by preventing oxidative stress-mediated activation of the ERK1/2–Cx43 signalling pathway.

Using an automated oxygen radical absorbance capacity assay, kiwifruit was shown to have greater antioxidant activity than grapefruit, apple or pear *in vitro*[38]. Increased antioxidant activity was also found in the plasma of human subjects fed kiwifruit juice, demonstrating the bioavailability of kiwifruit antioxidants in man[39]. The antioxidant activity of kiwifruit might play a role in the suppression of H_2O_2-induced phosphorylation of ERK1/2 and Cx43. This is supported by the superior free radical-scavenging activity of quercetin over BHT (Fig. 5). Together, these data suggest that the antioxidant properties of quercetin may be responsible for the suppression of H_2O_2-induced phosphorylation of ERK1/2 and Cx43 and prevention of GJIC inhibition.

In summary, kiwifruit extracts and quercetin alone prevented H_2O_2-induced inhibition of GJIC by blocking the oxidative stress-induced activation of the ERK1/2 and Cx43 signalling pathway. The present results suggest that the consumption of kiwifruit or adding quercetin as a dietary supplement might be an effective means to lowering the risk of developing cancer.

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All authors have contributed to the conception and design of the experiment, and acquisition of the data and their subsequent analysis and interpretation. Similarly, all authors have been involved in the critical revision of the paper and have approved the final version of the paper.

There are no conflicts of interest to declare by any of the authors.

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