Lack of Effect of the Flavonoids, Myricetin, Quercetin, and Rutin, on Repair of H_2O_2-Induced DNA Single-Strand Breaks in Caco-2, Hep G2, and V79 Cells

S. A. Aherne and N. M. O’Brien

Abstract: In the present study the effects of three flavonoids on the repair of H_2O_2-induced DNA strand breaks were investigated in Caco-2, Hep G2, and V79 cells. At the concentrations used, myricetin, quercetin, rutin, and H_2O_2 did not significantly affect cell viability in all the cell lines. Catalase activity was measured in V79 cells and was found to be considerably lower than activities previously measured in Caco-2 and Hep G2 cells. Cells were exposed to 50 µM H_2O_2 for 0.5 hour at 37°C. After treatment, DNA strand break repair in H_2O_2-treated cells was monitored at various time points over a 48-hour period using the alkaline single-cell gel electrophoresis assay. Caco-2 cells repaired faster than Hep G2 cells, which repaired considerably faster than V79 cells. Preincubation with 50 µM quercetin for 24 hours significantly decreased the extent of H_2O_2-induced DNA single-strand breaks throughout repair time points in Caco-2 cells (p < 0.05), but not in Hep G2 cells. Myricetin (50 µM) and rutin (50 µM) had no effect on repair in Caco-2 and Hep G2 cells. Preincubation for 10 hours with quercetin and rutin, but not myricetin, significantly decreased the initial extent of DNA damage induced by H_2O_2 in V79 cells (p < 0.05). However, from the results of this study, none of the three flavonoids increased the rate of repair of strand breaks in any of the cell types.

Introduction

Nonnutritive bioactive components found in fruits and vegetables have been investigated for their possible role in protection against and prevention of pathological diseases, such as cardiovascular disease and certain cancers (1–3). Epidemiological, in vivo and in vitro studies have suggested that naturally occurring plant compounds, such as the flavonoids, may inhibit and protect against various stages of the cancer process (1,2). The flavonoids occur predominantly in their glycosidic form in fruits, vegetables, cereals, legumes, beverages, nuts, and seeds. The most commonly occurring dietary flavonoids are those with hydroxylation on the 3’ and 4’ positions of the B-ring on the flavan nucleus, for example, myricetin, quercetin, and rutin (4,5).

The flavonoids exhibit a wide variety of biological activities, including antiviral, antibacterial, anti-inflammatory, anticarcinogenic, and antioxidant actions (2,6,7). By donating hydrogen atoms to peroxy radicals, the flavonoids terminate lipid peroxidation chain radical reactions (6,7). Several studies have shown the flavonoids to act as scavengers of superoxide anions, singlet oxygen, hydroxyl radicals, and lipid peroxy radicals (8–10). Thus the flavonoids are capable of quenching free radicals, which may promote mutations and may also protect DNA by interacting with carcinogens that have escaped detoxification processes (5). The flavonoids can also bind to metal ions, such as copper or iron (7,11,12), which are involved in many oxidation reactions. Flavonoids having a C-4 carbonyl group and a C-3 or C-5 hydroxyl group, such as quercetin and rutin, are capable of chelating iron ions (Figure 1) (13).

Transition metal ions, such as iron and copper, can react with H_2O_2 to produce other reactive oxygen species, such as hydroxyl radicals, in the Fenton reaction. H_2O_2 treatment has been shown to result in DNA damage, mutagenesis, sister chromatid exchange, and chromosome aberrations in mammalian cells in vitro (14–17). DNA alteration and repair represent an essential stage in the mechanism of carcinogenesis (18). Cells return to a normal state after proper repair, whereas improper repair of DNA damage can result in accelerated mutation rates and the appearance of tumors (19–21). Mechanisms of repair can differ significantly between organisms, cell types, and types of damage (22). When a cell containing damaged DNA replicates before the DNA is repaired, a permanent genetic alteration occurs that may eventually lead to carcinogenesis. As a consequence, cells that have a faster population-doubling time are more susceptible to carcinogenesis than slowly dividing cells, because there is less opportunity for DNA repair before cell division (23). Modulation and/or variation in cellular repair capacity may be studied at the cellular level, treating cells in vitro with a genotoxin, and during a period of incubation, monitoring removal of the induced damage (24).
Figure 1. Structures of 3 flavonols used in these experiments: myricetin, R1 = OH, X = OH; quercetin, R1 = H, X = OH; rutin, R1 = H, X = O-rutinose.

In a previous study, we demonstrated that the flavonoids are capable of protecting against H2O2-induced DNA strand breaks (17). The present study was carried out to determine whether the flavonoids had any effect on repair of H2O2-induced DNA strand breaks. In a recent study by Abalea and others (25), the effect of the flavonoid myricetin on repair of iron-induced DNA base oxidation in primary rat hepatocyte cultures was investigated. They reported that myricetin (100 µM) enhanced repair of iron-induced DNA base oxidation in the rat hepatocytes.

The cell models used in this study were human colonic adenocarcinoma Caco-2, human hepatocellular carcinoma Hep G2, and Chinese hamster lung V79 fibroblast cells. Caco-2 and Hep G2 cells have approximately the same population-doubling rates, whereas V79 cells have a faster rate of replication. The objectives of this study were 1) to observe the repair of H2O2-induced DNA single-strand breaks in Caco-2, Hep G2, and V79 cells and 2) to investigate whether preincubation with the flavonoids myricetin, quercetin, and rutin had any effect on repair of H2O2-induced DNA strand breakage in the three cell types.

Materials and Methods

Materials

Tissue culture materials, including Dulbecco’s modified Eagle’s medium (DMEM) (with L-glutamine and sodium bicarbonate), Williams’ E medium (with L-glutamine and without sodium bicarbonate), fetal calf serum, minimum essential medium nonessential amino acids, and trypsin (2.5 g/l) were purchased from Sigma Chemical (Poole, Dorset, UK). Myricetin, quercetin, and rutin were also purchased from Sigma Chemical. H2O2 of low mineral content was purchased from BDH (Poole, Dorset, UK). Myricetin, quercetin, and rutin were dissolved in DMSO, saline. For comparison, the incubation periods were made relevant to cell population-doubling times. Caco-2 and Hep G2 cells were supplemented with 50 µM myricetin, quercetin, or rutin for 24 hours, whereas the flavonoid supplementation period in V79 cells was 10 hours. After flavonoid preincubation, cells were washed three times with growth medium, and subsequent H2O2 exposure was in the absence of flavonoids and serum.

Incubation of Cells With Test Compounds

Myricetin, quercetin, and rutin were dissolved in DMSO, where the final concentration of the solvent in the culture medium was <0.07% (vol/vol). Control cells were exposed to the equivalent concentration of DMSO only, and results were not significantly different from those obtained from untreated cells. H2O2 was dissolved in phosphate-buffered saline. For comparison, the incubation periods were made relevant to cell population-doubling times. Caco-2 and Hep G2 cells were supplemented with 50 µM myricetin, quercetin, or rutin for 24 hours, whereas the flavonoid supplementation period in V79 cells was 10 hours. After flavonoid preincubation, cells were washed three times with growth medium, and subsequent H2O2 exposure was in the absence of flavonoids and serum. For oxidant treatment, all three cell lines were exposed to 50 µM H2O2 for 0.5 hour at 37°C.
ter oxidant treatment, cells were again washed with growth medium and were incubated with normal growth medium for a further 48 hours without flavonoids and H$_2$O$_2$.

Neutral Red Uptake Assay and FDA-EtBr Assay

The neutral red uptake assay, by a modification of the method of Hunt and associates (26), was carried out on cells supplemented with myricetin, quercetin, or rutin alone, cells supplemented with myricetin, quercetin, or rutin and then treated with H$_2$O$_2$ (repair Time 0), and cells at the final repair time point (48 h). Cytotoxicity was expressed as a percentage of cell viability relative to that of control cells. FDA-EtBr assay, an alternative index of cell viability, was carried out at all the repair time points by the method of Strauss (27). Twenty-five microliters of harvested cells were mixed with 25 µl of FDA-EtBr stain and kept at 37°C for five minutes. Twenty-five microliters of this mixture were pipetted onto a microscope slide and covered with a coverslip. Cells were counted under a blue filter on a Nikon Labophot fluorescence microscope, where green-stained cells are viable and red-stained cells are nonviable. Two hundred cells were counted per slide per condition. Viability was expressed as a percentage of the number of viable cells present relative to that of control cells.

ASCGE Assay

The ASCGE assay, by the method of Tice and associates (28), was used to assess the formation of DNA single-strand breaks in vitro. To investigate H$_2$O$_2$ dose responses, cells were exposed to increasing concentrations of H$_2$O$_2$ (10–100 µM) for 0.5 hour at 37°C in Caco-2, Hep G2, and V79 cells. After treatment, the extent of DNA strand break formation was assessed using the ASCGE assay. DNA repair was monitored by assessing the extent of DNA single-strand breaks at 0, 1, 3, 6, 24, and 48 hours after H$_2$O$_2$ treatment in Caco-2 and Hep G2 cells and 0, 0.5, 1.25, 2.5, 5, 10, 24, and 48 hours after H$_2$O$_2$ treatment in V79 cells. Application of the ASCGE assay as described is commonly used to monitor DNA single-strand break repair (24,29–32).

Microscope slides were precoated with 1% (wt/vol) normal-melting-point agarose at ~50°C in phosphate-buffered saline. When the agarose solidified, 1% (wt/vol) low-melting-point (LMP) agarose was pipetted onto the slides, immediately covered with coverslips, and put on ice to allow the agarose to solidify. Treated and nontreated cells were gently scraped, mixed with LMP agarose, and pipetted on top of the first LMP agarose layer on the slides. A third layer of LMP agarose was added to each slide. Slides were placed in cold lysis solution [2.5 M NaCl, 100 mM EDTA, 10 mM tris(hydroxymethyl)aminomethane, pH 10, 1% sodium sarcosinate], with 1% Triton X-100 and 10% DMSO added gently before each use, for 1.5 hours. Slides were then placed in a horizontal gel electrophoresis tank (Horizon 20-25, GIBCO BRL, Life Technologies, Paisley, Scotland) containing fresh electrophoresis solution (1 mM EDTA and 300 mM NaOH) for 40 minutes. Electrophoresis was carried out for 25 minutes at 4°C with a current of 25 V (300 mA) using a compact power supply. After electrophoresis, the slides were washed three times with neutralizing buffer [0.4 M tris(hydroxymethyl)aminomethane, pH 7.5] at 4°C for five minutes each. Slides were stained with EtBr (20 µg/ml) and covered with coverslips. Rate of strand break repair was estimated by calculating the slopes of the lines at the most rapid phase, which occurred between Time 0 and Hour 3 in all three cell lines.

Scoring

Cells were visualized using a Nikon Labophot fluorescence microscope. One hundred nuclei on each slide were visually scored from 0 (undamaged nucleus) to 4 (severely damaged nucleus) (29). Thus a negative control sample would have a score of 0, ranging to 400 for a maximally damaged sample. Samples were scored blind, and results were expressed as arbitrary units.

CAT Activity

CAT activity in cell sonicates was determined on the same day the cells were harvested by a modification of the method of Baudhuin and co-workers (33). This assay was performed at 25°C using 0.05 M potassium phosphate buffer, pH 7.5. CAT activity was measured at 465 nm using a Cary spectrophotometer (model 1E, Varian), where the remaining H$_2$O$_2$ was determined as a yellow “peroxy titanium sulfate.” One unit of CAT activity is defined as the amount of CAT that will decompose 1 µmol of H$_2$O$_2$ per minute at pH 7.5 and 25°C. Results are expressed as units of CAT activity per milligram of protein. Total protein was determined using the Bio-Rad microassay (34), where bovine serum albumin was the standard.

Statistical Analysis

DNA repair was monitored at several time points over a 48-hour period, and this was considered one repair experiment. Values are means ± SE of three independent repair experiments. Data were analyzed by one-way analysis of variance followed by the least significant difference test (35). The level of statistical significance was taken at $p < 0.05$.

Results

Effects of Flavonoids and H$_2$O$_2$ on Cell Viability

Caco-2, Hep G2, and V79 cells were incubated in the presence (50 µM) or absence of myricetin, quercetin, or rutin for various periods of time (24 h for Caco-2 and Hep G2 cells and 10 h for V79 cells). After incubation, the effect of
the flavonoids on percent cell viability was assessed using the neutral red uptake assay. None of the compounds were found to be toxic to the cells (data not shown). Caco-2, Hep G2, and V79 cells were exposed to 50 µM H2O2 for 0.5 hour in the absence of serum to determine the effects on cell viability. H2O2 did not significantly affect cell viability in all three cell lines (data not shown). Preincubation of Caco-2, Hep G2, and V79 cells with each flavonoid, followed by exposure to H2O2, did not significantly alter cell viability (repair Time 0). After treatment with H2O2, test medium was replaced with growth medium, and cells were incubated for a further 48 hours. Cell viability was determined at the 48-hour time point and did not significantly differ from control cells. As an example of the results obtained, cell viability in Hep G2 cells at the 0- and 48-hour repair times is shown in Table 1.

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<thead>
<tr>
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<th>% Cell Viabilityb</th>
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<tr>
<td></td>
<td>0 h</td>
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<tr>
<td>Controlc</td>
<td>100.0 ± 4.9</td>
</tr>
<tr>
<td>Myricetin</td>
<td>99.4 ± 3.7</td>
</tr>
<tr>
<td>Quercetin</td>
<td>94.2 ± 4.9</td>
</tr>
<tr>
<td>Rutin</td>
<td>104.2 ± 4.6</td>
</tr>
<tr>
<td>H2O2</td>
<td>104.9 ± 4.6</td>
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<td>NSd</td>
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| a: Values are means ± SE for 3 independent experiments. Hep G2 cells were preincubated with or without 50 µM myricetin, quercetin, or rutin for 24 h and then exposed to H2O2 (50 µM for 0.5 h at 37°C). b: Cell viability was assessed, using the neutral red uptake assay, 0 and 48 h after oxidant treatment. c: Control cells were not treated with flavonoids or H2O2. d: Statistical analysis was by one-way analysis of variance. NS, not significantly different from control cells.

Over the concentration range tested, V79 cells were the most sensitive to H2O2-induced DNA damage.

DNA Single-Strand Break Repair in Caco-2, Hep G2, and V79 Cells

Similar trends can be seen in all the repair curves (Figure 3). From 0 to 3 hours, there is a fast rate of repair followed by a much slower rate between 3 and 48 hours. At 48 hours, the extent of DNA single-strand breaks in Caco-2 and Hep G2 cells returned to background levels (68 ± 16 and 79 ± 3 arbitrary units for Caco-2 and Hep G2 cells, respectively), whereas considerably less repair occurred in V79 cells. The slopes of the repair curves (0–3 h) were calculated as an estimate of the rate of repair in each cell line. Repair of H2O2-induced DNA strand breaks occurred at a faster rate in Caco-2 cells than in Hep G2 and V79 cells (Figure 3).

Effect of Flavonoids on Repair of H2O2-Induced DNA Single-Strand Breaks

A set of control cultures, which were not treated with flavonoids or H2O2, were included in each repair experiment (negative control). The negative controls for Caco-2, Hep G2, and V79 cells were assessed for DNA strand breaks at the start (0 h) and end (48 h) of each repair experiment using the ASCGE assay. Over the 48-hour period, a slight increase in DNA single-strand breaks occurred in all three cell lines (Figures 4–6).

Caco-2 cells were preincubated with or without 50 µM myricetin, quercetin, or rutin for 24 hours and then exposed to H2O2 for 0.5 hour at 37°C. After oxidant treatment, test medium was replaced with normal growth medium. Over the following 48 hours, DNA strand break repair was monitored using the ASCGE assay (Figure 4). At repair Time 0,
24 hours of preincubation with myricetin, quercetin, or rutin significantly decreased H₂O₂-induced DNA single-strand breaks compared with cells treated with H₂O₂ only (positive control) \((p < 0.05; \text{Figure 4})\). At 1-, 6-, and 24-hour repair times, quercetin significantly decreased the extent of H₂O₂-induced DNA damage compared with the positive control \((p < 0.05; \text{Figure 4B})\). However, quercetin did not affect the rate of strand break rejoining compared with the positive control. Between 1 and 48 hours, myricetin and rutin had no effect on the rate of repair or extent of DNA single-strand breaks compared with H₂O₂-treated cells (\text{Figure 4, A and C}). DNA damage in Caco-2 cells returned to background levels after 48 hours in all treatment groups (\text{Figure 4}).

In Hep G2 cells, preincubation with 50 µM myricetin, quercetin, or rutin for 24 hours significantly decreased the extent of H₂O₂-induced DNA single-strand breaks \((p < 0.05)\) at repair Time 0 (\text{Figure 5}). Over the following 48 hours, myricetin, quercetin, and rutin had no significant effect on the extent of H₂O₂-induced DNA damage compared with the positive control. Quercetin and rutin did not affect the rate of repair compared with H₂O₂-treated cells, as estimated by calculating the slopes, whereas myricetin slightly, but nonsignificantly, decreased the rate of DNA strand break rejoining. V79 cells were supplemented with each flavonoid for 10 hours and then treated with H₂O₂ for 0.5 hour (\text{Figure 6}). At Time 0, quercetin and rutin significantly decreased H₂O₂-induced DNA single-strand breaks \((p < 0.05)\). From 0 to 1.5 hour, quercetin significantly decreased the extent of H₂O₂-induced DNA damage compared with the positive control \((p < 0.05; \text{Figure 6B})\). At 1.5 hours of repair, preincubation with myricetin and rutin also had a significant effect on the extent of DNA single-strand breaks in V79 cells \((p < 0.05)\). Between 2.5 and 24 hours, none of the flavonoids had a significant effect on the extent of H₂O₂-induced DNA damage. In addition, the flavonoids had no effect on the rate of repair as calculated from the slopes of the repair curves vs. the positive control. After 24 hours, H₂O₂-induced DNA single-strand breaks in V79 cells did not return to background levels \((57 ± 1 \text{ arbitrary units})\). DNA strand break repair was then assessed at 48 hours after H₂O₂ treatment, and the level of DNA single-strand breaks in V79 cells had not returned to negative control values (\text{Figure 3}).

**CAT Activity**

CAT activity was measured in V79 cells supplemented with or without 50 µM H₂O₂ for 0.5 hour at 37°C (\text{Table 2}). There was a slight but nonsignificant increase in CAT activity in H₂O₂-treated V79 cells compared with that of untreated cells.

**Discussion**

We recently showed that preincubation of Caco-2 and Hep G2 cells with myricetin, quercetin, or rutin for 24 hours protected against H₂O₂-induced DNA single-strand breaks in a dose-dependent manner (17). Our present aim was to determine whether the flavonoids myricetin, quercetin, and rutin had any effect on the repair of H₂O₂-induced DNA single-strand breaks. In this study, myricetin, quercetin, and rutin significantly decreased the initial extent of H₂O₂-induced DNA single-strand break formation in Caco-2 and Hep G2 cells \((p < 0.05; \text{Figures 4 and 5})\), confirming our previous findings. Quercetin and rutin, but not myricetin, also protected against initial H₂O₂-induced DNA single-strand breaks in V79 cells (\text{Figure 6}). The mechanism(s) by which the flavonoids are involved in protection against DNA damage is unknown; however, their ability to act as reactive oxygen species scavengers and metal ion chelators, carcinogen inactivators, modulators of enzyme activity, inhibitors of tumor cell growth, inducers of apoptosis, and modulators of DNA repair have been proposed (5,32). Oxidative DNA damage is subject to cellular repair processes, such as strand break rejoining and base excision repair (29). A recent study by Abalea and associates (25) showed the protective effects of the flavonoid myricetin on the repair of iron-induced DNA base oxidation products in primary hepatocytes. To the best of our knowledge, there have been no reports on the effects of flavonoids on DNA strand break
rejoining in vitro assessed using the ASCGE assay. Here, we report that myricetin, quercetin, and rutin did not significantly modulate the rate of DNA strand break rejoining in all three cell lines, even though quercetin significantly decreased the extent of DNA strand breaks throughout most of the repair period in Caco-2 cells (Figure 4).

Comparisons between H2O2-induced DNA single-strand breaks and consequent strand break rejoining in Caco-2, Hep G2, and V79 cells have not been previously reported. Of the three cell lines, V79 cells were the most sensitive to H2O2 (Figure 2), which has been reported in previous studies (36,37). Furthermore, Caco-2 (epithelial) cells repaired faster than Hep G2 (epithelial) cells, which repaired considerably faster than V79 (fibroblast) cells (Figure 3). The time taken to repair 50% of H2O2-induced DNA single-strand breaks was ~2.5 hours for Caco-2 cells, 6 hours for Hep G2 cells, and 24 hours for V79 cells. Originally, repair of H2O2-induced DNA single-strand breaks in V79 cells was monitored over a period of 24 hours. However, when repair had not returned DNA single-strand breaks to control levels, we

Figure 4. Effect of myricetin (A), quercetin (B), and rutin (C) on repair of H2O2-induced DNA single-strand breaks in Caco-2 cells. Cells were preincubated with (open circles) or without (open squares) 50 µM flavonoid for 24 h and then exposed to 50 µM H2O2 for 0.5 h at 37°C. After oxidant treatment, test medium was replaced with normal growth medium and cells were incubated for a further 48 h. Over this period of incubation, DNA repair was monitored by assessing extent of DNA damage at various time points using ASCGE assay. Control cultures (open triangles) were not treated with flavonoids or H2O2. Values are means ± SE for 3 independent experiments. Statistical analysis was by one-way analysis of variance followed by least significant difference test. *, Significantly different (p < 0.05) from H2O2-treated cells.
examined DNA repair 48 hours after H$_2$O$_2$ treatment. H$_2$O$_2$-induced DNA strand breaks did not return to background levels after 48 hours in this cell type (Figure 3).

Over the 48-hour period, a slight increase in DNA damage was seen in all three cell lines (Figure 2). This was possibly due to the development of a hyperoxic environment, as suggested by Collins and others (24), who also observed a small but consistent increase in DNA damage in untreated cells throughout repair experiments.

Variability in antioxidant defense or DNA repair, between cell types and growth state, could be important in determining the susceptibility of cells to genetic destabilization, cell death, or mutation (32). Previously, we measured CAT activity in untreated Caco-2 and Hep G2 cells (42.6 ± 9.3 and 36.6 ± 4.4 units/mg protein, respectively) (17). In the present study, CAT activity was lower in V79 cells than in the other two cell lines (26.5 ± 5.4 units/mg protein). CAT activity and H$_2$O$_2$ sensitivity have been inversely related where human lymphocytes had the lowest CAT activity and were the most H$_2$O$_2$ sensitive (38). Others believe that differences in H$_2$O$_2$ sensitivity in cells are due to different strand break repair capabilities, rather than variability.

Figure 5. Effect of myricetin (A), quercetin (B), and rutin (C) on repair of H$_2$O$_2$-induced DNA single-strand breaks in Hep G2 cells. Cells were preincubated with (open circles) or without (open squares) 50 µM flavonoid for 24 h and then exposed to 50 µM H$_2$O$_2$ for 0.5 h at 37°C. After oxidant treatment, test medium was replaced with normal growth medium and cells were incubated for a further 48 h. Over this period of incubation, DNA repair was monitored by assessing extent of DNA damage at various time points using ASCGE assay. Control cultures (open triangles) were not treated with flavonoids or H$_2$O$_2$. Values are means ± SE for 3 independent experiments. Statistical analysis was by one-way analysis of variance followed by least significant difference test. *, Significantly different ($p < 0.05$) from H$_2$O$_2$-treated cells.

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Figure 5. Effect of myricetin (A), quercetin (B), and rutin (C) on repair of H$_2$O$_2$-induced DNA single-strand breaks in Hep G2 cells. Cells were preincubated with (open circles) or without (open squares) 50 µM flavonoid for 24 h and then exposed to 50 µM H$_2$O$_2$ for 0.5 h at 37°C. After oxidant treatment, test medium was replaced with normal growth medium and cells were incubated for a further 48 h. Over this period of incubation, DNA repair was monitored by assessing extent of DNA damage at various time points using ASCGE assay. Control cultures (open triangles) were not treated with flavonoids or H$_2$O$_2$. Values are means ± SE for 3 independent experiments. Statistical analysis was by one-way analysis of variance followed by least significant difference test. *, Significantly different ($p < 0.05$) from H$_2$O$_2$-treated cells.
in antioxidant enzyme activities (32). The V79 cells in this study, which were the most H₂O₂ sensitive, had the lowest CAT activity and the lowest strand break rejoining capability. Hence, from our results, we report that sensitivity to H₂O₂ may be related to antioxidant enzyme activity and DNA repair capacity.

Initial damage to DNA is a prerequisite and initiation step to the process of carcinogenesis. Incomplete repair of this initial damage may result in uncontrolled cell proliferation, or progression (5). The anticarcinogenic actions of flavonoids may be attributed, in part, to their ability to protect against oxidative DNA damage and enhance the DNA repair process (25,39). Combining the results from our most recent (17) and present studies, we can conclude that the flavonoids protect against the initial step of carcinogenesis but do not modulate strand break rejoining processes in Caco-2, Hep G2, and V79 cells. In addition, we have shown that differences in antioxidant defense and DNA strand break rejoining processes among cell types reflect the susceptibility of cells to genetic alteration. Furthermore, we have demonstrated that variability in sensitivity to H₂O₂ may be related to CAT activity and DNA repair capability.

Figure 6. Effect of myricetin (A), quercetin (B), and rutin (C) on repair of H₂O₂-induced DNA single-strand breaks in V79 cells. Cells were preincubated with (open circles) or without (open squares) 50 µM flavonoid for 10 h and then exposed to 50 µM H₂O₂ for 0.5 h at 37°C. After oxidant treatment, test medium was replaced with normal growth medium and cells were incubated for a further 48 h. Over this period of incubation, DNA repair was monitored by assessing extent of DNA damage at various time points using ASCGE assay. Control cultures (open triangles) were not treated with flavonoids or H₂O₂. Values are means ± SE for 3 independent experiments. Statistical analysis was by one-way analysis of variance followed by least significant difference test. *, Significantly different (p < 0.05) from H₂O₂-treated cells.
Table 2. Catalase Activity in Chinese Hamster Lung V79 Fibroblasts

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<th>CAT Activity, b units/mg protein</th>
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<tr>
<td>Control c</td>
<td>26.45 ± 5.38</td>
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<tr>
<td>H₂O₂</td>
<td>31.44 ± 8.41</td>
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a: Values are means ± SE for 4 independent experiments.
b: V79 cells were exposed to 50 μM H₂O₂ for 0.5 h at 37°C. Catalase (CAT) activity was determined using a modification of the method by Baudhuin and others (45). 1 unit of CAT activity = 1 µmol of H₂O₂ removed per minute.
c: Control cells were not treated with H₂O₂.
d: Statistical analysis was by one-way analysis of variance. NS, not significantly different from control cells.

Acknowledgments and Notes

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