Dietary Flavones and Flavonoles Are Inhibitors of Poly(ADP-ribose)polymerase-1 in Pulmonary Epithelial Cells


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Abstract

The nuclear enzyme poly(ADP-ribose) polymerase-1 (PARP-1), which was initially known to be highly activated by oxidative stress-induced DNA strand breaks, has been shown to be involved in the pathophysiology of acute and chronic inflammatory diseases. PARP-1 deficiency in mice led to the discovery of its coactivating function in the nuclear factor-kappa B-mediated gene expression and in addition, pharmaceutical inhibition of PARP-1 was shown to reduce the production of inflammatory mediators. In this study, the in vitro PARP-1-inhibiting effect of various flavonoids was investigated. The flavonoids myricetin, tricetin, gossypetin, delphinidin, quercetin, and fisetin were identified as significant inhibitors of the purified enzyme. Further evaluation of these compounds in N-methyl-N-nitro-N-nitrosoguanidine-treated human pulmonary epithelial cells showed that the formation of the poly(ADP-ribose) polymers, as well as the decreased NAD\(^+\) levels, was reduced by quercetin, fisetin, and tricetin. Finally, IL-8 production of LPS-stimulated human pulmonary epithelial cells could be significantly reduced by these flavonoids. The results of this study indicate that specific flavonoids have PARP-1-inhibiting activity in addition to the earlier described antioxidant effects. PARP-1 inhibition and preservation of cellular NAD\(^+\) and energy production could play a role in the antiinflammatory activity of these specific flavonoids. In addition, these results indicate additional mechanisms by which flavonoids can exert antiinflammatory activity. Furthermore, these results indicate possibilities to use food-derived flavonoids in the treatment of chronic inflammatory diseases.


Introduction

The nuclear enzyme poly(ADP-ribose) polymerase-1 (PARP-1; EC 2.4.2.30), which catalyzes the formation of poly(ADP-ribose) (PAR) polymers from its substrate NAD\(^+\), has been described to be involved in the pathophysiology of both acute as well as chronic inflammatory diseases (1–5). PARP-1 is highly activated by oxidative stress-induced DNA strand breaks. Overactivation as a result of extensive DNA damage causes massive PAR polymer formation and consequently a rapid depletion of cellular NAD\(^+\) and ATP levels. If the resulting cellular energy crisis cannot be resolved, PARP-1 overactivation may lead to cell death.

Initially, the formation of the PAR polymers was known to facilitate repair of damaged DNA (6–8). Results of various studies indicate that the process of poly(ADP-ribosylation) may also facilitate transcriptional activity (9). The formation of the negatively charged PAR polymers and the transfer of these polymers to acceptor proteins like histones initiate electrostatic repulsion between histones and DNA. The subsequent remodeling of the chromatin architecture enhances the accessibility of genes for the transcriptional machinery and thus enhances transcription (9). More recently, PARP-1 has also been shown to be involved in the regulation of the nuclear factor-kappa B (NF-kB)- and activator protein 1 (AP-1)-mediated transcription of inflammatory cytokines and chemokines (10–12). Pharmaceutical inhibition of PARP-1 was demonstrated to have beneficial effects in various animal models for inflammatory diseases such as endotoxin shock or pulmonary inflammation (1,13). The aim of this study was to evaluate dietary flavonoids for their PARP-1-inhibiting activity. Flavonoids are polyphenolic compounds found in fruits and vegetables and plant-derived products like red wine and tea and can be divided into different subclasses such as anthocyanidins, flavonols, and flavones. The intake of dietary flavonoids has been related to a reduced risk for several
Genistein was obtained from Alexis. Naringenin, kaempferol, delphinidin and cyanidin were obtained from Extrasynthese. Dimethoxyflavone, and 3,5,7,3'-tetrahydroxychalcone, fustin, tricetin, gossypetin, 3'-pentamethoxyflavone were obtained from ICC. Results of the enzyme assay were further evaluated for their PARP-1 inhibiting activity in MNNG-treated human lung epithelial cells. Treatment of these cells with 25 μmol/L MNNG during 30 min in the presence or absence of flavonoids, added 15 min before the MNNG treatment. The synthetic PARP inhibitors 3-AB and PJ34 were used as positive controls. After the incubation, the cells were trypsinized, washed with PBS, and fixed in methanol. After fixation, cells were stained for PAR polymers as previously described (20). At least 100 cells per slide were evaluated for the presence of PAR polymers in their nucleus using fluorescence microscopy and Lucia GF 4.80 software (Nikon). Subsequently, the percentage of PAR polymer positive cells was calculated.

**Materials and Methods**

**Materials.** Naringin, fisetin, rutin, morin, quercetin, taxifolin, (+)-catechin, myricetin, phenol, hydroquinone, resorcinol, Tween 20, acetone, 3,7,5',5'-tetramethoxybenzylidine, hydrogen peroxide (H2O2), 3-aminozobenzamide (3-AB), MNNG, bovine serum albumin, DMEM, and LPS (O26:86) were all obtained from Sigma. Biacalein, 4,2',4'-trihydroxychalcone, fustin, tricetin, gossypetin, 3',5,7-trihydroxy-3,4'-dimethoxyflavone, and 3,5,7,3',4'-pentamethoxyflavone were obtained from ICC. Delphinidin and cyanidin were obtained from Extrasynthese. Genistein was obtained from Alexis. Naringenin, kaempferol, β-NAD+, and 1,4-dithiothreitol were obtained from MP Biomedicals. Catechol was obtained from Janssen Chimica. Human rPARP-1 and biotinylated NAD+ were purchased from Trevigen. HBSS, fetal bovine serum, trypsin, and penicillin/streptomycin were all obtained from Invitrogen Life Technologies. N-(6-oxo-5,6-dihydro-phenanthridin-2-yl)-N,N-dimethylacetamide (PJ34) was obtained from Merck. Peroxidase-labeled streptavidin was purchased from Zymed. Polyvinylchloride microtiter plates were obtained from BD Biosciences. The 10H hybridoma was kindly provided by Dr. M. Miwa, via Riken Cell Bank, Tsukuba Institute of Medical Research. The cell supernatant containing mouse monoclonal anti-PAR polymer antibody 10H was produced by Dr. W. Buurman (University of Maastricht). FITC-conjugated goat anti-mouse immunoglobulin and fluorescent mounting medium were obtained from DAKO. Peroxidase-labeled streptavidin was purchased from Zymed. Polyvinylchloride microtiter plates were obtained from BD Biosciences. The 10H hybridoma was kindly provided by Dr. M. Miwa, via Riken Cell Bank, Tsukuba Institute of Medical Research. The cell supernatant containing mouse monoclonal anti-PAR polymer antibody 10H was produced by Dr. W. Buurman (University of Maastricht). FITC-conjugated goat anti-mouse immunoglobulin and fluorescent mounting medium were obtained from DAKO.

**PARP-1 inhibition ELISA.** The capacity of the compounds to inhibit PARP-1 was first determined using an enzyme inhibition assay described by others (18,19), with minor modifications as previously described (20). In short, human rPARP-1 was incubated in a 96-well microtiter plate with a reaction mixture containing 50 μmol/L β-NAD+ (10% biotinylated β-NAD+, 90% unlabeled β-NAD+), 1 mmol/L 1,4-dithiothreitol, and 1.25 mmol/L nicked DNA. The formation of the PAR polymers was detected with peroxidase-labeled streptavidin and 3,3',5,5'-tetramethoxybenzylidine. PARP-1 activity was expressed as absorbance at 450 nm. PARP-1 inhibition of flavonoids was evaluated by addition of these compounds to the reaction mixture. The type of PARP-1 inhibition, specific or nonspecific, by flavonoids was analyzed in incubations of PARP-1 and putative inhibitors with various concentrations of β-NAD+. After which Lineweaver Burk plots were constructed.

**Cell culture.** A549 lung epithelial cells were grown at 37°C in a humidified 5% CO2 atmosphere and were cultured in DMEM with 4.5 g/L glucose, 10% fetal bovine serum, 2 mmol/L glutamine, 50,000 units/L of penicillin, and 50 mg/L of streptomycin.

**Cellular NAD+ assay.** PARP-1 activation in cultured cells was measured as previously described (20). MNNG was used to induce DNA strand breaks, activate PARP-1, and deplete intracellular NAD+ levels. Cells were incubated in a 96-well microtiter plate with 25 μmol/L MNNG during 30 min in the presence or absence of flavonoids, added 15 min before the MNNG treatment. The flavonoids used were not cytotoxic, as demonstrated by the LDH leakage test with incubation periods up to 24 h (data not shown) (21). To minimize a possible indirect protective effect of the flavonoids via scavenging of hydroxyl radicals and reduced formation of DNA strand breaks, the alkylating agent MNNG was used instead of H2O2 in this assay to induce PARP-1 activity. The PARP-1 inhibitor 3-AB strongly prevented the decrease in NAD+ levels and served in all experiments as a positive control. Intracellular NAD+ was determined using the NAD+-cycling method based on the method from Jacobsen and Jacobsen (22).

**Immunohistochemical staining of PAR polymers.** To confirm the PARP-1 inhibiting effects of flavonoids, we measured the formation of PAR polymers. A549 cells (~0.8 × 106 cells/well in a 6-well plate) were treated with MNNG for 5 min in the presence or absence of flavonoids, which were added 15 min before the MNNG treatment. The synthetic PARP inhibitors 3-AB and PJ34 were used as positive controls. After the incubation, the cells were trypsinized, washed with PBS, and fixed in methanol. After fixation, cells were stained for PAR polymers as previously described (20). At least 100 cells per slide were evaluated for the presence of PAR polymers in their nucleus using fluorescence microscopy and Lucia GF 4.80 software (Nikon). Subsequently, the percentage of PAR polymer positive cells was calculated.

**LPS treatment and IL-8 measurement.** A549 cells were treated with 100 μg/L LPS for 16 h in the absence or presence of flavonoids, which were added 30 min before the LPS treatment. After incubation, medium was collected and centrifuged (2000 × g; 10 min at 4°C) and supernatant was stored at −80°C until measurement of IL-8 with ELISA kits (CLB/Sanquin). Cells were trypsinized and processed for immunohistochemical staining of the PAR polymer as described above.

**Statistics.** Experiments were performed in triplicate and results are reported as means ± SEM. The effect of the various flavonoids on MNNG-induced NAD+ depletion and PAR polymer formation and LPS-induced IL-8 release compared with the condition without flavonoids was statistically analyzed using ANOVA followed by Dunnett’s post hoc testing. Differences were considered to be statistically significant if P < 0.05.

**Results**

**PARP-1 enzyme inhibition.** To determine the extent of PARP-1 inhibition by several flavonoids, human rPARP-1 was incubated with the flavonoids (100 μmol/L) for 1 h (Table 1). PARP-1 was potently inhibited by the flavonoids myricetin (93%) and tricetin (80%). Also, flavonoids gossypetin (73%), delphinidin (62%), quercetin (62%), and fisetin (60%) were found to be strong PARP-1 inhibitors. Biacalein, naringin, or (+)-catechin showed <10% inhibition. We evaluated the phenolic compounds phenol, catechol, resorcinol, and hydroquinone for their PARP-1-inhibiting activity, which showed 8, 12, 9, and 5% inhibition, respectively. The methylated metabolites of quercetin, 3',5',7-trihydroxy-3,4'-dimethoxyflavone and 3,5,7,3',4'-pentamethoxyflavone, had weak or no PARP-1 inhibition, whereas 4,2',4'-trihydroxychalcone and rutin had no inhibition at all. For the flavonoids myricetin, tricetin, quercetin, and fisetin, which showed the most potent PARP-1-inhibiting activity, Lineweaver Burk plots were constructed using various concentrations of NAD+ Inhibition of PARP-1 by myricetin, quercetin, and fisetin was a mixed type, with competitive as well as noncompetitive characteristics. Inhibition of PARP-1 by tricetin was mostly competitive (data not shown).

**Effects on MNNG-induced NAD+ depletion.** Specific flavonoids that showed the most significant inhibition in the enzyme assay were further evaluated for their PARP-1-inhibiting activity in MNNG-treated human lung epithelial cells. Treatment of these cells with 25 μmol/L MNNG activated PARP-1 and induced depletion of cellular NAD+ levels to ~23% of control levels after 30 min. MNNG-induced depletion of intracellular NAD+ was determined using the NAD+-cycling method based on the method from Jacobsen and Jacobsen (22).
NAD\(^+\) stores was used as a parameter of PARP-1 activation. Depletion of the NAD\(^+\) levels was significantly attenuated in the presence of the flavonoids quercetin, fisetin, and tricetin at a concentration of 100 \(\mu\)mol/L (Fig. 1). Delphinidin, gossypetin, myricetin, and morin, which also showed significant inhibition of the purified enzyme, failed to prevent the MNNG-induced decrease in NAD\(^+\) levels (data not shown).

**PAR polymer formation.** To confirm the PARP-1-inhibiting effect of the flavonoids quercetin, fisetin, and tricetin, A549 cells were treated with MNNG in the presence of these flavonoids.

Subsequently, the formation of PAR polymers was detected using immunocytochemical staining. Due to the very rapid catabolism of PAR polymers by poly(ADP-ribose) glycohydrolase (PARG) (9), incubation time of MNNG was reduced to 5 min. In control cells, no PAR polymer formation was observed. Treatment of the cells with MNNG induced a massive increase in the number of PAR polymer positive cells (24%). Preincubation of the cells with quercetin, fisetin, and tricetin for 15 min dose-dependently inhibited PAR polymer formation (Fig. 2B), indicating that the attenuated depletion in NAD\(^+\) levels was primarily due to direct PARP-1 inhibition. The synthetic PARP-1 inhibitors PJ34 and 3-AB were included as positive controls and PJ34 almost completely reversed PAR polymer formation (Fig. 2B).

**Effects on LPS-induced IL-8 production.** The flavonoids quercetin, fisetin, and tricetin, which attenuated both the decrease in NAD\(^+\) levels as well as the formation of PAR polymers, were further studied for their effect on LPS-induced IL-8 production in A549 cells. Quercetin and fisetin as well as tricetin significantly reduced IL-8 production (Fig. 3A). The synthetic PARP-1 inhibitor PJ34 showed a similar antiinflammatory activity (Fig. 3B). Immunohistochemical staining of PAR polymers was applied to determine PARP-1 activation during LPS treatment of A549 cells. No detectable PAR polymer formation could be measured in the A549 cells after treatment with 100 \(\mu\)g/L LPS for 24 h after stimulation (data not shown).

**Discussion**

In this study, the flavonoids quercetin, fisetin, and tricetin significantly inhibited the nuclear enzyme PARP-1. This inhibition was

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**TABLE 1** Characteristics and PARP-1 inhibition of flavonoids tested in this study

<table>
<thead>
<tr>
<th>Flavonoid</th>
<th>Class</th>
<th>Hydroxylation pattern</th>
<th>Methoxylation pattern</th>
<th>C2-C3 pattern</th>
<th>Percent PARP-1 inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>4',2',4'-Trihydroxychalcone</td>
<td>Chalcone</td>
<td>4',2',4'</td>
<td>+</td>
<td>n.i.</td>
<td></td>
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<tr>
<td>Baicalin</td>
<td>Flavone</td>
<td>5,6,7</td>
<td>+</td>
<td>5 ± 4</td>
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<tr>
<td>Naringenin</td>
<td>Flavanone</td>
<td>5,7,4'</td>
<td>—</td>
<td>21 ± 3</td>
<td></td>
</tr>
<tr>
<td>Naringenin(^1)</td>
<td>Flavanone</td>
<td>5,4'</td>
<td>—</td>
<td>2 ± 0</td>
<td></td>
</tr>
<tr>
<td>Genistein</td>
<td>Isoflavone</td>
<td>5,7,4'</td>
<td>+</td>
<td>14 ± 4</td>
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<tr>
<td>Kaempferol</td>
<td>Flavonol</td>
<td>3,5,7,4'</td>
<td>+</td>
<td>27 ± 4</td>
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<tr>
<td>Fisetin</td>
<td>Flavonol</td>
<td>3,7,3',4'</td>
<td>+</td>
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<td>Morin</td>
<td>Flavonol</td>
<td>3,5,7,2',4'</td>
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<td>42 ± 7</td>
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</tr>
<tr>
<td>Quercetin</td>
<td>Flavonol</td>
<td>3,5,7,3',4'</td>
<td>+</td>
<td>62 ± 3</td>
<td></td>
</tr>
<tr>
<td>Rutin(^2)</td>
<td>Flavonol</td>
<td>5,7,3',4'</td>
<td>+</td>
<td>n.i.</td>
<td></td>
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<tr>
<td>Taxifolin</td>
<td>Flavanone</td>
<td>3,5,7,3',4'</td>
<td>—</td>
<td>20 ± 2</td>
<td></td>
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<tr>
<td>(+)-Catechin</td>
<td>Flavonol</td>
<td>3,5,7,3',4'</td>
<td>—</td>
<td>4 ± 4</td>
<td></td>
</tr>
<tr>
<td>Cyanidin</td>
<td>Anthocyanidin</td>
<td>3,5,7,3',4'</td>
<td>—</td>
<td>20 ± 3</td>
<td></td>
</tr>
<tr>
<td>Tricetin</td>
<td>Flavone</td>
<td>5,7,3',4',5'</td>
<td>+</td>
<td>80 ± 0</td>
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<tr>
<td>Myricetin</td>
<td>Flavonol</td>
<td>3,5,7,3',4',5'</td>
<td>+</td>
<td>93 ± 3</td>
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<tr>
<td>Delphinidin</td>
<td>Anthocyanidin</td>
<td>3,5,7,3',4',5'</td>
<td>—</td>
<td>62 ± 4</td>
<td></td>
</tr>
<tr>
<td>Gossypetin</td>
<td>Flavone</td>
<td>3,5,7,8,3',4'</td>
<td>+</td>
<td>73 ± 4</td>
<td></td>
</tr>
<tr>
<td>3',5,7'-Trihydroxy-3,4'-dimethoxyflavone</td>
<td>3',5,7,3',4'</td>
<td>+</td>
<td>13 ± 2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3,5,7,3',4'-Pentamethoxyflavone</td>
<td>3,5,7,3',4'</td>
<td>+</td>
<td>n.i.</td>
<td></td>
<td></td>
</tr>
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</table>

\(^1\) Naringen is a naringenin glycoside with a rhamnoglucoside group at position 7.
\(^2\) Rutin is a quercetin glycoside with a rhamnoglucoside group at position 7.
\(^3\) n.i., no inhibition.
\(^4\) PARP-1 activity was measured after incubating 400 \(\mu\)g/L PARP-1 for 1 h in the presence of 1.25 mg/L nicked DNA, 50 \(\mu\)mol/L NAD\(^+\), and 100 \(\mu\)mol/L flavonoid at 4°C.
observed not only with the purified PARP-1 enzyme but also in MNNG-treated human pulmonary epithelial cells. Furthermore, a dose-dependent decrease in IL-8 production was observed after LPS treatment of the A549 cells in presence of quercetin, fisetin, and tricetin. These data indicated that their PARP-1 inhibiting activity may contribute to antiinflammatory effects via inhibition of NF-κB-mediated gene expression.

The inducible transcription factor NF-κB plays an important role in the inflammatory and immune response and regulates the production of pro-inflammatory cytokines and chemokines such as IL-8 (23). PARP-1 has been reported to be a coactivator of NF-κB (10,11). However, whether PARP-1 enzyme activity or the protein itself is required for complete activation of NF-κB is still a matter of debate (24,25). Nevertheless, pharmaceutical PARP-1 inhibitors were successfully applied in various in vitro and in vivo models of inflammation and were found to reduce the production of nitric oxide (NO) and pro-inflammatory cytokines such as TNFα, IL-6, and IL-8 (1,5,13,26).

The precise mechanisms through which flavonoids inhibit the production of pro-inflammatory cytokines remain to be elucidated. Their antioxidant properties are in general believed to be primarily responsible for the antiinflammatory effects. Reactive oxygen species (ROS) have been described to be involved in the pathology of inflammatory diseases (27,28). In addition, ROS have been described to induce activation of redox-sensitive transcription factors such as AP-1 and NF-κB (29), subsequently leading to increased production of several inflammatory mediators and chemokines, including IL-8. In various studies, the inhibiting effects of flavonoids on the production of pro-inflammatory markers were observed. Reduced production of pro-inflammatory cytokines, inducible nitric oxide synthase (iNOS) expression, and NO production have been observed after treatment with the flavonoids quercetin, apigenin, and luteolin in LPS- or phorbol myristate acetate-stimulated cultured cells (30–32). These effects have been found to be associated with inhibition of the NF-κB pathway via reduced phosphorylation of IκBα and reduction of the nuclear translocation and DNA binding of NF-κB (30–32). In addition, these flavonoids attenuated the production of pro-inflammatory mediators via regulation of the transcription factor AP-1 (31). In this study, specific flavonoids had significant PARP-1 inhibiting activity. In an attempt to assess PARP-1 activation during LPS treatment of A549 cells, we applied immunocytochemical staining of the cells at various time points, ranging from 5 min to 24 h after treatment. However, no PAR polymer formation could be detected in these cells, in contrast to cells treated with MNNG. This may be due to the application of a mild stimulus (100 μg/L LPS) that most likely failed to induce high levels of ROS and measurable PAR polymer formation. In addition, PAR polymers are very rapidly catabolized by PARG (9), which may also result in nondetectable PAR polymer formation. After stimulation of A549 cells with IL-1β and TNFα, Erdelyi et al. (33) also did not detect PAR polymer formation in contrast to the massive PAR polymer formation observed in H2O2-treated cells (33). Whether inhibition of PAR polymer formation is the mechanism by which flavonoids reduce IL-8 production in LPS-treated A549 cells is still inconclusive.

In this study, inhibition of PARP-1 by flavonoids was not solely a competitive inhibition. Although inhibition of PARP-1 by tricetin was competitive, myricetin, quercetin, and fisetin showed a mixed type of inhibition. These results indicate that PARP-1 activity was not solely reduced by an interaction of these flavonoids with the catalytic site of PARP-1 but also by nonspecific interactions of the flavonoids with PARP-1. Because it was also suggested by others that the presence of the enzyme and not the enzymatic activity of PARP-1 is required for complete NF-κB activation (25), nonspecific binding of flavonoids to

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PARP-1 could probably prevent interaction of PARP-1 with NF-κB and subsequently reduce the NF-κB-mediated gene expression.

When tested with the purified enzyme, the flavonoids myricetin, tricetin, gossypetin, delphinidin, quercetin, and fisetin clearly inhibited PARP-1 at 100 μmol/L (∼ 60%), whereas other flavonoids such as kaempferol and naringenin showed considerably lower PARP-1 inhibiting activity. Given that in foods most flavonoids occur predominantly as β-glycosides and upon ingestion become extensively metabolized into glucuronidated, sulfated, or methylated conjugates (34,35), a number of commercially available conjugated flavonoids were screened for their PARP-1 inhibiting activity. The addition of methyl groups to flavonoids like quercetin drastically reduced the inhibiting activity. 3',5',7-Trihydroxy-3,4'-dimethoxyflavone showed only weak inhibition compared with quercetin and the inhibiting activity of 3,5,7,3',4'-pentamethoxyflavone was even more reduced. Glycosylation as in rutin and naringin considerably decreased inhibition compared with the aglycones quercetin and naringenin. Evaluating the PARP-1 inhibiting activity of phenolic compounds such as phenol, catechol, resorcinol, and hydroquinone indicated that the position of the hydroxy groups (ortho, meta, or para) did not clearly contribute to the PARP-1 inhibiting activity of these compounds. However, the presence of the C2-C3 double bond seemed to influence the PARP-1 inhibiting activity of some flavonoids, because both quercetin and fisetin showed substantially higher PARP-1 inhibiting activity compared with the flavonoids taxifolin and fustin.

Although myricetin showed the strongest PARP-1 inhibiting activity in the enzyme assay, it failed to significantly inhibit PARP-1 in the A549 cells. The Lineweaver Burk plot of myricetin indicated that the inhibition of the purified PARP-1 enzyme by myricetin showed both competitive as well as noncompetitive characteristics, indicating that myricetin also showed nonspecific protein binding, which might reduce the intranuclear concentration. Furthermore, several nonspecific interactions between flavonoids and proteins have been reported (36,37). Because PARP-1 is located in the nucleus and PARP-1 inhibitors first need to pass the cell membrane and enter the nucleus to inhibit the enzyme, nonspecific interactions could decrease the final intracellular and nuclear concentration of myricetin or other flavonoids. Alternatively, the flavonoids quercetin and fisetin, which also showed mixed type of inhibition similar to myricetin, did prevent the MNNG-induced PAR polymer formation and decreased NAD^+ levels. This suggests that these flavonoids pass the cell membrane and enter the nucleus in sufficient amounts to exert PARP-1-inhibiting effects.

Because PARP-1 is also involved in facilitating DNA repair, complete inhibition of PARP-1 appears undesirable. For that reason, mild inhibition would be the preferred method of action. The flavonoids quercetin, fisetin, and tricetin inhibited PARP-1, both in the enzyme assay as well as in A549 cells. By mildly inhibiting the enzyme activity of PARP-1, these flavonoids would protect against NAD^+ and ATP depletion, reducing the risk of cell death-induced inflammation. Moreover, the reduction of LPS-induced IL-8 release at similar levels indicated an antiinflammatory effect via NF-κB. Furthermore, a low level of PARP-1 activity would allow for repair of DNA damage. In addition, it was previously observed that administration of the potent synthetic PARP-1 inhibitor INO-1001 in a porcine model of thoracic aortic cross-clamping-induced ischemia/reperfusion injury did not impair DNA repair (38). DNA damage in peripheral blood mononuclear cells was evaluated by the COMET assay, both in vivo as well as ex vivo, and the INO-1001-treated group and vehicle-treated group did not differ. Also, expression of the cyclin-dependent kinase inhibitor gene p27 in the kidney was evaluated. Expression of p27 increased equally in both the vehicle-treated and INO-1001-treated group and it was suggested that DNA damage and repair was not impaired by treatment with the PARP-1 inhibitor INO-1001 (38).

Apples, grapes, onions, and ginkgo biloba are dietary sources of the PARP-1-inhibiting flavonoids quercetin, fisetin, and tricetin (39). In industrialized societies such as Western Europe and the United States, the estimated intake of flavonoids and flavones reportedly ranges from 20 to 34 mg/d (40–42). The repeated intake of food supplements containing high doses of flavonoids (e.g. 1 g quercetin/d) has been shown to increase plasma concentrations, reaching levels in the low micromolar range (43–45). This implies that the observed in vitro effects were achieved at concentrations that may be reached in vivo after the use of supplements.

Specifically in the treatment of chronic inflammatory diseases, such as chronic obstructive pulmonary disease (COPD), long-term use of dietary supplements is promising, because no effective treatment has been developed yet. In COPD patients, we previously reported increased oxidative stress and increased inflammatory cytokines, including IL-8, which were accompanied by increased percentage of PAR polymer positive peripheral blood mononuclear cells, indicating a chronic and systemic PARP-1 activation (3). As a consequence of chronic PARP-1 activation, NAD^+ turnover will be increased, implying an increased demand on ATP production for resynthesis of NAD^+. This increased turnover of NAD^+ and demand for energy may contribute to exercise intolerance and muscle weakness, which greatly impair the quality of life of COPD patients. Reduction of oxidative stress and inhibition of PARP-1 by food-derived compounds might reduce this chronic state of energy consumptive cycles and ameliorate systemic inflammatory conditions. Dietary flavonoids like quercetin, fisetin, and tricetin would be potential candidates, not only based on their antioxidant effects but also based on their PARP-1 inhibiting as well as antiinflammatory effects as described in this study.

**Literature Cited**


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