Prevention of Cellular ROS Damage by Isovitexin and Related Flavonoids

Chun-Mao Lin[1,2], Chien-Tsu Chen[2], Hsiao-Hui Lee[1], Jen-Kun Lin[1]

Abstract

The antioxidant properties of isovitexin and related flavonoids were studied. Isovitexin inhibited xanthine oxidase with an IC_{50} value of = 15.2 μM. The flavonoid analogues, apigenin, kaempferol, quercetin, myricetin, and genistein also inhibited xanthine oxidase with IC_{50} values of 0.58, 2.18, 1.09, 9.90, and 4.83 μM, respectively. Isovitexin protected DNA from the Fenton reaction-induced breakage in a dose-dependent manner with an IC_{50} value of 9.52 μM. Isovitexin also protected HL-60 cells from the ROS damage induced by the xanthine/xanthine oxidase reaction. Isovitexin exhibited the lowest cytotoxicity toward HL-60 cells (LD_{50} >400 μM) compared to the other flavonoids examined. In addition, excess hydrogen peroxide induced by cadmium in A2780 ovarian cells was significantly suppressed by isovitexin. These results suggest that isovitexin in rice may protect cells from oxidative stress.

Flavonoids are widely distributed in various species of plants. Numerous groups have investigated the antioxidant properties of flavonoids [1]. Many are also potent inhibitors of several enzymes, such as xanthine oxidase, cyclooxygenase, and lipooxigenase [2], [3]. Rice is the main daily diet in Asia. *Oryza sativa* is widely cultivated, and flavonoids isolated from rice have been described and characterized [4]. A major property of flavonoids is their ability to scavenge hydroxyl radicals. All of the flavonoids tested in our study could scavenge hydroxyl radicals, but isovitexin, a glycosyl flavonoid, was the most effective. The antioxidation activity of isovitexin is comparable to those of α-tocopherol and ascorbic acid. The related flavonoid compounds, apigenin, kaempferol, quercetin, myricetin, and genistein (Fig. **1**) were chosen for comparison with isovitexin. The cytotoxicity and antioxidative activites of the tested flavonoids are listed in Table **1**. Apigenin was most toxic to HL-60 cells with a LD_{50} value of 35 μM after 48 h of treatment. Isovitexin had the lowest cytotoxic effect on HL-60 cell with a LD_{50} value of greater than 400 μM. The selected flavonoids were also evaluated for their ability to reduce lipid peroxidation induced by the Fenton reaction. All the selected flavonoids inhibited xanthine oxidase. Isovitexin displayed the highest IC_{50} value (15.2 μM) towards xanthine oxidase compared with the other flavonoids. Apigenin (IC_{50} = 0.58 μM) had a comparable inhibitory activity as allopurinol (0.65 μM). Isovitexin

Affiliation: [1] Institutes of Biochemistry and Molecular Biology, National Taiwan University, Taipei, Taiwan · [2] Department of Biochemistry, College of Medicine, Taipei Medical University, Taipei, Taiwan

Correspondence: Prof. Jen-Kun Lin · Institutes of Biochemistry and Molecular Biology, · College of Medicine, National Taiwan University · No. 1, Section 1 · Jen-Ai Road · Taipei · Taiwan · Phone: +886–2-2356–2213 · Fax: +886–2-2391–8944 · E-Mail: jklin@ha.mc.ntu.edu.tw

Received: April 30, 2001 · **Accepted:** October 6, 2001

Bibliography: Planta Med 2002; 68: 365–367 · © Georg Thieme Verlag Stuttgart · New York · ISSN 0032-0943

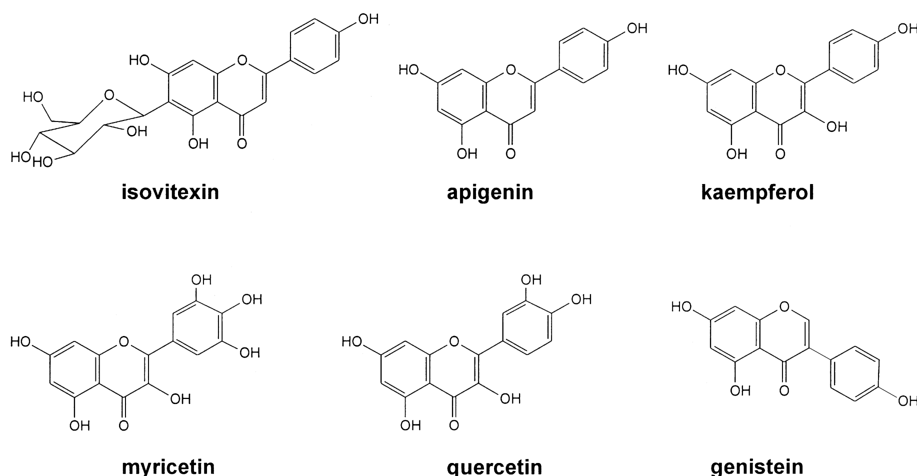

Fig. **1** Structures of flavonoids.

Table **1** Cytotoxicity and antioxidative properties of flavonoids

Compound	***Cytotoxicity LD_{50} (μM)***	***Inhibition of lipid peroxidation* (%)***	***Xanthine oxidase Inhibition IC_{50} (μM)***
apigenin	35.2 ± 7.3	63.5 ± 3.5	0.58
kaempferol	38.6 ± 5.4	87.2 ± 8.1	2.18
quercetin	44.2 ± 4.6	82.4 ± 6.2	1.09
myricetin	67.5 ± 7.1	87.2 ± 4.5	9.90
genistein	73.8 ± 6.6	35.4 ± 2.3	4.83
isovitexin	> 400	94.2 ± 5.1	15.2
α-tocopherol+	ND	100**	ND
ascorbate+	ND	93.6 ± 5.6+	ND
allopurinol+	ND	ND	0.65

ND: not determined.
* : 5 μM of each compound was used in lipid peroxidation assay.
**: Inhibitory effect of α-tocopherol was assigned as 100%.
+: Positive controls.

was a weaker inhibitor of xanthine oxidase compared to apigenin. The sugar moiety of isovitexin may hinder competition of the active site of the enzyme.

Hydroxyl radical (·OH) generated by the Fenton reaction is known to cause oxidatively induced DNA strand breaks to yield open circular DNA (relaxed circular DNA). Hydroxyl radical scavengers can protect DNA from strand breaks induced by hydroxyl radical. The hydroxyl radical scavenger ability of isovitexin was evaluated. Supercoiled DNA migrates faster than relaxed circular DNA on agarose gel electrophoresis. DNA strand breakage was induced *in vitro* in the presence of H_2O_2 and Fe^{2+} (lane 4, Fig. **2A**), while DNA in the presence of H_2O_2 or Fe^{2+} alone did not show significant strand breakage (lanes 2 and 3, Fig. **2A**). Treatment of DNA with isovitexin reduced the concentration of relaxed circular DNA in a dose-dependent manner (lanes 5 – 10, Fig. **2A**) with an IC_{50} value of 9.52 μM. The xanthine/xanthine oxidase (X/XO) reaction is considered to be an important biological source of reactive oxygen species (ROS) that contributes to oxidative stress in many pathological processes. HL-60 cells were treated with 50 μM xanthine and 5.0 U/L xanthine oxidase, and the resulting cell apoptosis was characterized using sub-G1 peak quantification by flow cytometry with propidium iodine staining. Flow cytometry showed that 65.9% cells underwent apoptosis after X/XO treatment for 15 h. Cells were rescued from ROS induced apoptosis by the presence of isovitexin; 49.6% of cells underwent apoptosis in the presence of 100 μM isovitexin, but only 18.6% of cells underwent apoptosis in the presence of 200 μM isovitexin (Fig. **2B**). This result indicates that isovitexin with low cytotoxicity (LD_{50} > 400 μM), but with significant xanthine oxidase inhibitory activity and antioxidative activity, exhibited potent protection of cells from xanthine/xanthine oxidase induced damage.

Among the several possible mechanisms for metal genotoxicity, oxidative damage has been intensively studied in recent years. Cadmium induced production of ROS associated with apoptosis was also reported [5], [6]. Green DCH fluorescence is generated when DCF-DA is hydrolyzed by esterase and oxidized by hydrogen peroxide. The FL1-H fluorescence intensity on a FACScan flow cytometer reflects the hydrogen peroxide concentration in living cells. The hydrogen peroxide concentration of A2780 cells was elevated upon heavy metal cadmium treatment (250 μM)

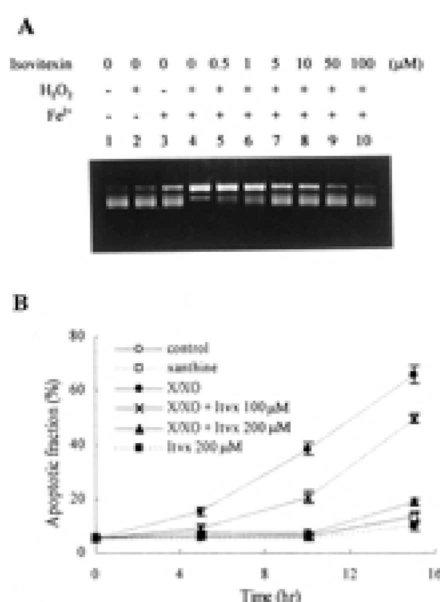

Fig. **2** Isovitexin suppressed ROS induced DNA strand breakage and cell injury. Plasmid DNA was incubated at 37 °C for 30 min with 100 mM H_2O_2 and 50 μM ferrous sulfate in the presence or absence of isovitexin (**A**). Isovitexin protection of HL-60 cells from ROS induced apoptosis by the xanthine/xanthine oxidase reaction (**B**).

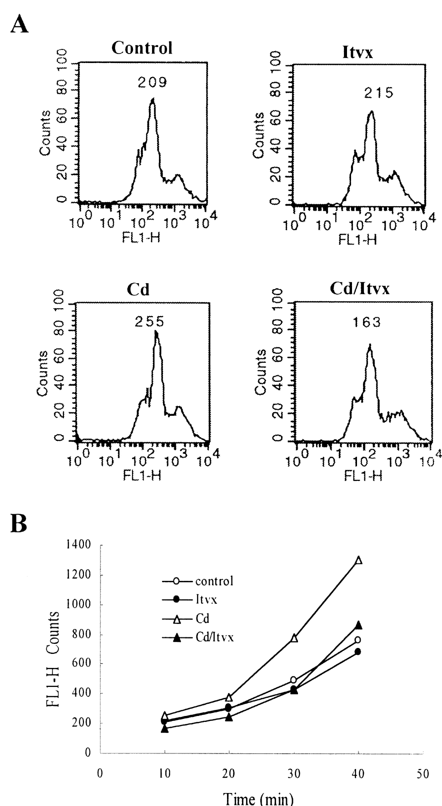

Fig. **3** Isovitexin suppression of cadmium-induced production of hydrogen peroxide. A2780 ovarian cells were untreated, or treated with isovitexin (100 μM), cadmium (250 μM), or cadmium and isovitexin. Cells were stained with DCF-DA and subjected to flowcytometry. The peaks of FL1-H fluorescence intensity are indicated for each treatment (**A**). The fluorescence intensity during 40 min of treatment is shown in **B**.

for 10 min. Isovitexin added prior to cadmium treatment reduced the concentration of hydrogen peroxide in living cells (Fig. **3A**). Fig. **3B** shows the hydrogen peroxide content in cells after various treatments over 40 min. The FL1-H intensity of untreated cells increased 3.5-fold in 40 min, whereas the intensity of cells treated with isovitexin and cadmium increased 2.8-fold and 5.1-fold, respectively. The FL1-H intensity of cadmium treated cells was suppressed by isovitexin. These results suggest that high intake of dietary rice containing isovitexin may help protect against heavy metal toxicity.

Materials and Methods

Materials: The isovitexin was purified from rice *Oryza sativa* as described previously. The spectral and physical characteristics (IR, ^{1}H-NMR, ^{13}C-NMR, FAB-MS) of purified isovitexin were identical to the published data [7]. Apigenin, kaempferol, quercetin, myricetin, and genistein were purchased from Sigma (St. Louis, MO).

Biological activities: Lipid peroxidation was measured by the thiobarbituric acid (TBA) assay as described [4]. Xanthine oxidase activity was measured spectrophotometrically by determining uric acid formation at 295 nm with xanthine as substrate [8]. Supercoiled DNA-relaxation assay was carried out by determining pUC-19 plasmid DNA strand breaks induced by the Fenton reaction after 30 min incubation at 37 °C in the presence or absence of isovitexin. Protection of HL-60 cells from xanthine/xanthine oxidase reaction induced cell apoptosis by isovitexin was assessed. The apoptotic fraction, taken as the sub-G1 peak after propidium iodine (PI) staining of cells was determined by flow cytometry (Becton Dickinson) [9]. Hydrogen peroxide of A2780 ovarian cells upon cadmium treatment (250 μM) was determined using DCF-DA staining, and then analyzed using a FL1-H wavelength band of FACScan (Becton Dickinson) as described [10].

Acknowledgements

This study was supported by the National Science Council, NSC 89–2320-B-002–223 and by the National Health Research Institute, NHRI-GT-Ex89B913L.

References

1 Metodiewa D, Kochman A, Karolczak S. Evidence for antiradical and antioxidant properties of four biologically active *N, N*-diethylaminoethyl ethers of flavanone oximes: a comparison with natural polyphenolic flavonoid (rutin) action. Biochemistry & Molecular Biology International. 1997; 41: 1067–75
2 Hayashi T, Sawa K, Kawasaki M, Arisawa M, Morita N. Inhibition of cow's milk xanthine oxidase by flavonoids. Journal of Natural Products 1988; 51: 345–8
3 Nagao A, Seki M, Kobayashi H. Inhibition of xanthine oxidase by flavonoids. Bioscience, Biotechnology & Biochemistry 1999; 63: 1787–90
4 Tamogami K, Kodama O. Coronatine elicits phytoalexin production in rice leaves (*Oryza sativa* L.) in the same manner as jasmonic acid. Phytochemistry 2000; 54: 689–94
5 Liu F, Jan K. DNA Damage in arsenite- and cadmium-treated bovine aortic endothelial cells. Free Radical Biology & Medicine 2000; 28: 55–63
6 Stohs SJ, Bagchi D, Hassoun E, Bagchi M. Oxidative mechanisms in the toxicity of chromium and cadmium ions. J. Environ. Pathol. Toxicol. Oncol. 2000; 19: 201–13
7 Ramarathnam N, Osawa T, Namiki M, Kawakishi S. Chemical studies on novel rice hull antioxidants. 2. Identification of isovitexin, a *C*-glycosyl flavonoid. J. Agric. Food Chem. 1989; 37: 316–9
8 Chang WS, Chang YH, Lu FJ, Chiang HC. Inhibitory effects of phenolics on xanthine oxidase. Anticancer Res. 1994; 14: 501–6
9 Inoue M, Suzuki R, Koide T, Sakaguchi N, Ogihara Y, Yoshisada Y. Antioxidant, gallic acid, induces apoptosis in HL-60RG. Biochemical & Biophysical Research Communications 1994; 204: 898–904
10 Ubezio P, Civoli F. Flow cytometric detection of hydrogen peroxide production induced by doxorubicin in cancer cells. Free Radical Biology & Medicine 1994; 16: 509–16