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Cisplatin and thio-compound

Cisplatin binding DNA to generate superoxide and hydoxyl radical is proposed due to the formation of platinum (Pt)-DNA complex which iron ion catalyzes the reaction; whether platinum reacting with hydrogen peroxide to generate Fenton like reaction or platinum only promoting the Fenton reaction still need more work. The cytotoxicity induced by cisplatin could be reduced by antioxidants. Glutathione depletion was observed on the effects of cisplatin where could be reduced by reacting with lipoate or amifostine. The chloride of cisplatin could exchange with thio-group of lipoate or amifostine to form a complex; meanwhile, selenium also form a $(NH_3)_2Pt(SeO_3)$ complex to reduce the toxicity of cisplatin. The structure of those complex will still need some experiments to illustrate a highly capability to induce the generation of free radicals.

Isovitexin and iNOS

Isovitexin and related flavonoids isolated from rice hull of *Oryza sativa* have been identified with strong antioxidant activity. We investigate the effect of isovitexin on the activities of inducible cyclooxygenase (COX-2) and inducible nitric oxide synthase (iNOS) in lipopolysaccharide (LPS)-activated RAW 264.7 macrophages. Isovitexin's antioxidant activity was proved to be a potent protection against DNA damage induced by Fenton reaction. Moreover, isovitexin significantly scavenged the excess hydrogen peroxide induced by LPS. Treatment of cells with isovitexin markedly reduced LPS-stimulated NO and prostaglandin production, in a concentration-dependent manner, with an IC_{50} of about 58.5 and 80.0 μM, respectively. The expression of both iNOS and COX-2 were also inhibited by isovitexin treatment. Transient transfection experiment showed that isovitexin suppressed iNOS promoter and NF-κB-dependent transcriptional activities. In Addition, western blotting analysis revealed that isovitexin prevented degradation of IκB-alpha and translocation of NF-κB from the cytoplasm into the nucleus. Our results clearly demonstrate that suppression of ROS-mediated NF-κB activity by isovitexin may be beneficial in reducing the development of inflammation.

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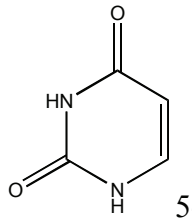
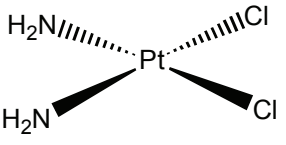
Cisplatin and thio-compound

` { S ⁄ W S , ~ ‰ F ⁄ ˘ · " v ł ¡ A , g ª ƒX(anti-neoplastic) ´ S M, ~‰F ^ ˜ " « alkylating agent ¡A¥ H · S C † › ¦M·r £ " ł fi ˜ ¡A 5-FU (5-fluorouracil) 'M Cisplatin (cis-diamminedichloroplatinum (II), DDP) « K ‹ Ø @ → ¥ ¿ † ˜ ⁄ @ ‰ " ƒ ' X ¦ C – q ƒ ¥ · ™ ´ " ‹ ª ¤ s ⁄ W A „ L ¥ h S › ™ „ Œ 5-FU » ‡ H { B fi ƒ ¢ ¥ ⁄ ˘ † ¡ A ¤ ª ƒ ‹ ¥ ' fi æ fi ` fi õ S K ™ fl S » ⁄ O ¡ F ƒ ¤ Cisplatin " " „ Œ ¯ p A Cisplatin · C ⁄ ¶ £ ¥ ƒ í ¶ W ¥ (¡ E O 2 ¯) ⁄ ¿ † B ł æ ƒ ¥ ¡ A † £ ¥ ˝ † › M r ¡ ' C ¤ " ⁄ S X ¥ ¡ ' A M S ‚ ¿ … — " " ⁄ ⁄ ł £ ƒ A ƒ b † › M ⁄ " ¯ ª M ¥ \ ‹ ‹ ' Ł ¡ A ¤ † › M ¥ ˝ " « · ⁄ W " ¥ \ fl ¡ A ' ‹ ‹ ª s ¡ F ¥ ˝ ⁄ ˘ ⁄ W ¡ A ⁄ l ‹ ⁄ ¥ S @ S ł , p A « E « ¥ › Þ " – e · Q ¡ C



5-FU



Cisplatin

5-FU ‹ O ` { S ⁄ W ł · ¶ „ M ¤ ˘ ¥ ¿ " " ⁄ @ " S ¥ N ` ć S ‹ , ƒ ‰ ¡ F A ƒ ˜] « ¡ A S ¤ › † " c » P " " „ Œ ¯ o 5-FU ¤ ª ƒ ł « O ¯ @ ¥ fi æ ƒ A S ` K f ! » ˜ ¤ ¤ fi æ ƒ B ¥ " " S » " " fl ⁄ O ¡ C £ ƒ P " ¿ @ « 5-FU » P 0.35% $H_2O_2$ » P 40μM " " ¯ K ´ ⁄ 37¢J 30 ⁄ ˜ > ˘ ¡ A ¥ h fi æ fi ` (fl DNA) " " ⁄ ø q ⁄ ˘ (fragmentation) · ⁄ ¡ C ƒ b « ƒ P @ « ⁄ U (2.0mM) ¡ A 5-FU (⁄ · ƒ ł 70% ¶ W ` ł – « ‹ ‰ Ł Ø ¯ @ ¥ fi æ fi ` fi » ˜ ¡ A S K ' ¤ ¤ fi æ ƒ B ¥ " " S » æ Phenylalanine (30%) ƒ ¡ C 5-FU ¥ ¤ ª S fl ‰ Ł „ I fi æ ˘ S @ ¥ ¡ A malondialdehyde (MDA) † £ ¥ 5-FU ¿ @ " " … W [¡ A ƒ b ¿ @ ‹ 10mM fi ¡ A linolenic acid fi æ ⁄ ˘ {« › S ‹ 60% ¡ C ¯ ¥ 5-FU " " ¤ ⁄ ƒ ⁄ ¶ W ¥ X S ¡ ‰ F ˜ · " v ł S S ¥ N ` ´ S ¡ ‰ ª F ˜ C ¥ ¡ A « ł " , › « • ‰ Æ " ⁄ " • s M ¥ ´ † z " " , ˜ ¡ C ¥ « e S › ¥ ¿ ƒ b ı ƒ ¥ » « ƒ ~ p e ¡ A – · Q 5-FU , g Fenton reaction " " † £ " « mw144 ‹ O ƒ ¡ H ⁄ " R 5-FU ¶ i ƒ Fenton reaction " " † £ " « A ф { ƒ b ⁄ † " ¤ „ Œ ¯ ⁄ ¡ A " fl d fi ¶ ¡ ‹ « 6.06 ⁄ ˜ ` ¡ A • s ¥ ´ ƒ ¤ " « H Fe(II) " " ¿ @ … W [ƒ … W [¡ C fi ‹ …h " R ‰ Ł ^ » (GC/MS) ⁄ " R ¡ A ¥ ´ ƒ ¤ " « " fl d fi ¶ ¡ ‹ 7.20 ⁄ ˜ ` ¡ A ⁄ ⁄ l ¶ q 144 (mw144) ¡ C ^{1}H NMR " " ¯ ⁄ ˘ ф { C_6-H H ⁄ ˘ † fi ¶ ¡ " " … W [ƒ · ⁄ ¡ A ^{13}C NMR ¥ X † { • s ¥ ´ ƒ ¤ ⁄ E _ 77.5 » P 152 " fi p ¡ C fi ⁄ ⁄ I ⁄ l fi p (Molecular ion peak) " " ⁄ ¥ ¡ " R ¡ A ¥ ´ ƒ ¤ " « mw144 " † c ¥ i fl ‹ 5FU-epoxide ($C_4N_2HO_3F$) ' ¿ 5,6-dihydroxyluracil (diOHU, $C_4N_2H_4O_4$) ' ¿ N-formyl-N-oxoacetyl urea (FOU, $C_4N_2H_4O_4$) ¡ A ¤ ‹ ^ S ƒ ¥ » P S ¥ N ˘ S ¥ \ fl ~ ‰ F S – · Q M ¶ I i f ⁄ ⁄ ¡ C

Cisplatin » P ¥ h fi æ fi ` fi C » ˜ o † ¥ ´ ¶ W fi æ ƒ ¥ (¡ E O 2 ¯) ⁄ ¿ † B ł æ ƒ ¥ ¡ A † ¥ ´ ƒ ¥ ⁄ S ¤ † Q » ¡ A Cisplatin » P DNA † ƒ ¤ ¿ ƒ ¤ platinum (Pt) » P DNA ‰ ˘ ƒ X ¯ Ø ¡ A » ⁄ of ¥ † £ ¥ ˝ ¡ A ¯ K ´ ⁄ ¶ ° ⁄ ˘ ¤ ⁄ ˘ † ¡ A O S _ , g ¥ platinum » P „ I fi æ ⁄ ˘ † B (H_2O_2) o ¥ ´ Fenton-like reaction ¡ A ' ¿ platinum ¶ ¨ ¥ [– j Fenton- reaction " " – j « ¤ ^ ⁄ £ † M ¡ ¡ C † › M · ł ° ¥ i , g ¥ antioxidants ¡ A f p vitamin C ¡ B curcumin ¡ B N-acetylcysteine S @ ¥ ¿ ƒ · » · ¡ A « ¥ X Cisplatin " " S @ ¥ ¿ ¤ " " ‰ T » ⁄ of ¥ † £ ¥ ˝ ¡ C Platinum analogs ⁄ ˘ ƒ X " « ⁄ S » ⁄ of ¥ ¡ A ¥ ¥ Cisplatin ⁄ S ^ ƒ ⁄ ˘ ƒ X " « Carboplatin „ Œ ^ „ Œ ¡ C

ф { Cisplatin " " S @ ¥ ¿ – ƒ æ H glutathione depletion ¡ A ⁄ ⁄ › ¥ K ¥ [lipoate ' ¿ amifostine (¤ " S S free thiol) ¡ A ¥ i · ⁄ glutathione depletion ¡ A f] ƒ „ – · Œ cisplatin " " · ´ ⁄ l • | » P S t thio ⁄ ˘ ƒ X " ™ " -SH ¥ · « ¡ A S ¿ ƒ ¤ ¿ ø ⁄ ˘ ƒ X " « GS-Pt ¡ B Lipoate-Pt ¡ B ' ¿ WR1065-Pt [32] ¡ C ¿ ø ⁄ ˘ ƒ X " ™ " ¥ ´ ƒ ¤ ¡ A ‹ O S _ ¤ ƒ ł " « » ⁄ of ¥ " " fl ⁄ O ¡ A ⁄ £ ‹ ˜ † M ¡ ¡ F ¯ ª M ł o › ł ¤ ¤ fi k þ glutathione ¤ ƒ " " , ‹ r ¤ ¡ A f] ł y ƒ ¤ glutathione · ⁄ ¡ A „ I fi æ ⁄ ˘ † B Ł k † M £ ¡ A l ‰ Ł „ I fi æ ⁄ ˘ … W [[33] ¡ A ¥ i fl ‹ O glutathione

› §¡A¤˘Glutathione Peroxidase Lglutathione ¥ ¸ ' † M £ „ L fiæ/˘†B' ›P¡C

« e › z † { ¶ H ¥ ‡Qglutathione “”Glutathione Reductase / § » † ƒ] / l·´ §Cl•| glutathione “”• ¥¢[34]¡A¤ › ƒ]¥ i fl ©¥glutathione “”` › ¡A' ¸‹O · ´/»lP Cisplatin § ƒ¤ $(NH_3)_2Pt(SeO_3)$¿ø/˘ƒ X«¡ A ›§C ‡\“”fi æ˘ £C[35]¡A¥ fil ƒ]ƒ „ §C Cisplatin “”‹ ‡°¡A› †z /]£†M• ¡¡C Cisplatin “”§@¥ ¸/ / » / o ƒ ¥ ¡A¯K£¥˝l¶°/˘¤ / ˘‡“” ¤ ¡A» n´†M“”‹O ¤ ” / @ › / ˘ ‡ ” ø/? oƒ ¥ †£¥˝

Isovitexin and iNOS

The toxicity ascribed to the superoxide radical is believed caused by superoxide's direct interaction with biological targets. Reactive oxygen species (ROS) can initiate a wide range of toxic oxidative reactions [1]. Oxidative stress has been implicated in an enormous variety of physiological and pathological processes, including aging, cancer, diabetes mellitus, atherosclerosis, neurological degeneration and arthritis. Antioxidant treatment can prevent the organ injury associated with ROS [2,3]. Hydrogen peroxide is a mediator of inflammation via NFκB pathway activation [4]. Prostaglandin biosynthesis and nitric oxide production have been implicated in the process of carcinogenesis and inflammation. Prostaglandins, especially prostaglandin E_2 (PGE_2), affect cell proliferation, tumor growth, and suppress the immune response to malignant cells. Therefore, high levels of prostaglandins could favor malignant growth [5]. Two isozymes, designated COX-1 and COX-2, have been identified, and each are encoded by separate genes. The COX-1 isozyme is believed to be a housekeeping protein in most tissues and seems to catalyze the synthesis of prostaglandins for normal physiological functions. COX-1 is expressed at essentially constant levels and does not fluctuate in response to various stimuli. In contrast, COX-2 is rapidly induced by tumor promoters, growth factors, cytokines, and mitogens in various cell types [6]. Many cell types associated with inflammation, such as macrophages, endothelial cells and fibroblasts, express the COX-2 gene upon induction [7]. Nitric oxide (NO) is released by a family of enzymes, constitutive NO synthase and an inducible NO synthase (iNOS), with the formation of stoichiometric amounts of L-citrulline from L-arginine. iNOS-mediated excessive and prolonged NO generation has attracted attention because of its relevance to epithelial carcinogenesis. NO has been reported to cause mutagenesis and deamination of DNA bases and to form carcinogenic *N*-nitrosoamines [8]. With respect to the role of NO in the post-initiation phase, it should be noted that it plays important roles in such inflammatory responses as edema formation and hyperplasia, as well as in papilloma development in mouse skin. NO is also involved in the production of VEGF, the overexpression of which induces angiogenesis, vascular hyperpermeability, and accelerated tumor development [9]. NO can stimulate tumor growth and metastasis by promoting the migratory and invasive abilities of tumor cells, which may also be triggered by activation of cyclooxygenase (COX)-2. Treatment of TPA in mice led to edema and papilloma formation by enhancing COX-2 protein expression. Specific COX-2 inhibitors could counteract these biological events. Suppression of enzyme induction and the activities of iNOS/COX-2 is a new paradigm for the prevention of carcinogenesis in several organs. Thus, selective inhibitors of iNOS, COX, or both may have a therapeutic role in certain cancers [10].

The active form of NF-κB is composed mainly of two proteins: p65 and p50. In unstimulated cells, NF-κB exists in the cytosol in a latent form bound to its inhibitory protein, IκB. After stimulating the cells with various agents, IκB becomes phosphorylated and subsequently succumbs to proteolytic degradation. Releasing IκB from NF-κB complex allows the activation and nuclear accumulation of the active NF-κB subunits [11,12]. The NF-κB family of transcription factors regulates expression of many genes, including the iNOS gene, involved in immune and inflammatory responses. The promoter region of the murine gene encoding iNOS contains two NF-κB binding sites, located at 55 and 971 base pairs upstream from the TATA box [13]. The binding of the potentially relevant transcription factor, NF-κB, to the κB sites has been shown to be functionally important for iNOS induction by LPS [14]. At least three different signal transduction pathways to trigger iNOS mRNA expression in 3T3 cells were reported. All these pathways seem to converge in the activation of the essential transcription factor, NF-κB

[15-17]. It has been proposed that reactive oxygen species are involved in the activation of NF-κB via regulation of various redox-sensitive protein kinases or tyrosine kinases [18-20]. Antioxidants such as (-)-epigallocatechin-3- gallate (EGCG) [21], resveratrol [22], aspirin [23] and naturally occurring flavonoids [24], have been reported to suppress NO production, and their inhibition mechanisms are based on their ability to inhibit the activation of NF-κB.

Rice possesses special dietary importance in Asia, where the incidence of breast and colon cancer is markedly lower than that in the western world [25]. Some investigations of potential beneficial effects of specific rice constituents exhibiting effects on prevention or amelioration of malignant disease have been conducted [26]. It has been reported that rice constituents counteract chemical-induced mutagenicity, tumor promotion, and carcinogenicity [27,28]. However, little is known about which specific molecules may be responsible for these activities. A number of mechanisms have been proposed by which such agents suppress tumors, but they all culminate in interference with the proliferation and survival of initiated cells [29]. Constituents from rice bran have been found to be beneficial for cancer prevention by epidemiological survey. Rice bran contains several classes of chemopreventive agents, *e.g.,* flavonoids and their glycosides, tocotrienols, and γ-oryzanol. Isovitexin and related flavonoids were isolated from rice hull of *Oryza sativa* and identified to have strong antioxidant activity [30,31]. Furthermore, xanthine oxidase inhibition, DNA protection, and prevention of heavy-metal-induced cell damage by isovitexin were described in our laboratory [31]. Here, we further evaluate the antioxidative properties of isovitexin, and examine the production of PGE_2 and NO from mouse macrophages. The molecular mechanisms of the reduction of iNOS and COX-2 expression and NF-κB activation in LPS-stimulated RAW 264.7 cells were further explored. The present data suggest that isovitexin could protect against endotoxin-induced inflammation by suppression of COX-2 and iNOS activation.

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Cisplatin and thio-compound

ƒbDNA fragmentation „ Œ ¯, Cisplatin H ¿ @ « W¥ DNA " " Fenton reaction † £ ¥ ˝ † B DNA " " {« ⁄ £ ƒ Fenton reaction " " † B Cisplatin ' glutathione " " Glutathione Reductase ⁄ § » † ƒ] ⁄ l · ´ ⁄ l • | > § C glutathione " " • ¥ ¢ ¡ A ¤ > ƒ] ¥ i fl < ¥ O [- j glutathione " " ^ > ¡ A ' ‚ < O · ´ ⁄ > l P Cisplatin § ƒ ¤ $(NH_3)_2Pt(SeO_3)$ ¿ ø ⁄ ƒ X ¡ " A » § C ¤ ‡ \ " " fiæ ⁄ ˘ £ ⁄ O A ¥ fl ƒ] ƒ „ § C Cisplatin " " < ‡ ° ¡ A ⁄ W z > † z ' | « l ‹ Œ Cisplatin " " § @ ¥ ⁄ ƒ ¥ G » ⁄ o ƒ ¥ † ¡ £ A ¥ K ´ ⁄ l ¶ ° ⁄ ˘ ¤ ⁄ ˘ ‡ (Masuda et al,1998 ¡ F Radhakrishna et al, 1998) " " ¤ ¤ ^ £ ƒ b ¥ » „ Œ ¯ , ˘ [„ - q » > ń ¡ ‡ M Cisplatin " " platinum ⁄ ˘ ‡ ƒ ¥ G ¥ » ⁄ o ƒ ¥ † ¡ £ A ¥ ˝ ƒ ¤ DNA fragmentation fl ‚ a DNA ¡ A ¥ fl , g ¥ DNA † ƒ X § ‚ ƒ ¤ Pt-DNA ‰ ˘ ƒ X ¯ Ø ¡ C GS-Pt ¡ B Lipoate-Pt ¡ B WR1065-Pt ' ‚ $(NH_3)_2Pt(SeO_3)$ ¥ ¿ ø ⁄ ƒ X " " § ƒ ¤ ¡ A ' < ¯ † w ¡ C ‡ o ⁄ ˘ ‡ ¤ ¥ i fl < ‡ O fl ´ " " „ q ⁄ l ¥ ˝ ƒ ¤ „ « (electron generator) ¡ A ¥ u > n ƒ ‡ A • " " – ¤ " ¡ A ¥ i ¥ H § ƒ ¤ ƒ Ù ƒ ¥ ¡ A ƒ] < Platinum analogs » ⁄ o ⁄ £ ¶ ¨ † ¥ ˝ ¶ W i æ ƒ ¥ (¡ E O_2^-) ⁄ ‚ † B æ ƒ ¥ " " ¥ ˝ ƒ ¤ ¡ F ƒ b pH2.8 ⁄ ‚ 8.5 fi ¥ » ⁄ o tryptophan ƒ ¥ (Zang and Rodgers, 1999) ¡ C ¯ K ´ ⁄ l ¶ ° ⁄ ˘ Cisplatin » ⁄ o ƒ ¥ † " ¥ ˝ ‡ ¡ A ¥ fl „ I fiæ ⁄ ˘ † B » P ¤ ⁄ ¡ A ° Fenton-like reaction ¡ A < † M ¥ " " ⁄ ˘ ‡ ¡ A „ I fiæ ⁄ ˘ † B < O § _ ¥ ¿ ¯ K ´ ⁄ l < O ⁄ @ > ¥ … " ¡ ^ Œ

Isovitexin and iNOS

Protection of supercoiled DNA from hydroxyl radical damage by isovitexin.

Hydroxyl radical (·OH) generated by the Fenton reaction is known to cause oxidative induced DNA strand breakage to yield an open circular DNA (relaxed circular DNA) under biological conditions. The hydroxyl radical scavenger property of isovitexin or related flavonoids on reducing the supercoiled DNA strand breakage caused by hydroxyl radical was evaluated. Fig. 2A is the result obtained from an agarose gel of pUC-19 plasmid DNA that was subjected to Fenton reaction in the absence or presence of flavonoids. Supercoiled DNA migrates faster than relaxed circular DNA in agarose gel electrophoresis. DNA strand breakage could be induced *in vitro* in the presence of H_2O_2 and Fe^{2+} (lane 4). Treatment of DNA with the ascorbic acid, α-tocopherol, or flavonoids was tested, and the percentage of relaxed form DNA was quantified in a lower panel. These results showed that the percentage of relaxed form DNA was reduced from 83.6% to 42.5% in the presence of isovitexin, which is the most effective agent among the test compounds for reducing DNA relaxation. Genistein, quercetin, and kaempferol showed comparable antioxidative activity in this assay system. These results suggest that isovitexin reacted with and/or scavenged the hydroxyl radicals and reduced the DNA damage in the tested system.

Isovitexin lowers LPS-activated cellular hydrogen peroxide in Raw 264.7 macrophage cells

Green DCH fluorescence is generated when DCF-DA is hydrolyzed by esterase and is 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 Raw 264.7 cells was elevated upon LPS treatment (100 ng/ml) for 30 min. Isovitexin added prior to LPS treatment reduced the concentration of hydrogen peroxide in living cells (Fig. 2B). The FL1-H intensity of untreated cells increased in 30 min upon LPS treatment, whereas the LPS-induced intensity of isovitexin-treated cells (5, 10, 20 μM respectively) was suppressed in a dose-dependent manner. Isovitexin, with the ability to lower LPS-activated cellular hydrogen peroxide, possesses the potential to reduce oxidative stress in RAW 264.7 macrophage cells**.**

Isovitexin decreased LPS-induced NO production and iNOS expression in activated macrophages

The half-life of NO is very short, so we used nitrite production as an indicator of NO released in LPS-activated macrophage to investigate the anti-inflammatory effects of isovitexin. The concentration-response relationships were determined 18 h after treatment with LPS

(50 ng/ml) alone or in the presence of isovitexin. Isovitexin was found to reduce NO generation in a concentration dependent manner (Fig. 3A). Isovitexin reduced NO generation with an IC_{50} about 58.5 μM. Cell viability assay verified that the inhibition by isovitexin was not due to general cellular toxicity (data not shown). The isovitexin dissolved in DMSO did not interfere with the Griess reaction. The protein levels of iNOS upon isovitexin treatments in activated macrophage cells were evaluated. RAW 264.7 cells maintained under normal conditions express slightly detectable levels of iNOS protein. After stimulation with LPS, the level of iNOS protein level increased. Inhibition of iNOS protein by isovitexin was detected in a concentration-dependent manner after 18 h treatment. The relative level of iNOS protein after treatment with 25, 50, 75, and 100 μM isovitexin was 0.86, 0.64, 0.38, and 0.13, respectively, versus LPS alone (Fig. 3B). The β-actin with constant expression was an internal control. The inhibitory concentration for iNOS protein expression was similar to that for the reduction of nitrite formation. In order to investigate whether the suppression of iNOS protein by isovitexin was due to reduced iNOS mRNA expression, a RT-PCR analysis for total mRNA samples, extracted from RAW 264.7 cells after 8 h treatment, was carried out. The amplification of cDNA with primers specific for mouse iNOS and GAPDH (as control gene) is shown in Figure 3C. The results indicat that lower levels of iNOS mRNA were expressed in the presence of isovitexin in LPS-activated macrophages. The data suggest that isovitexin modulated iNOS expression at the transcriptional level. Consistent with previous findings, isovitexin suppressed iNOS gene expression, thus inhibiting the production of NO in LPS-stimulated RAW 264.7 cells. To further investigate the importance of LPS and isovitexin in modulating expression of iNOS, transient transfection was performed using mouse iNOS promoter-drived luciferase construct. LPS-induced iNOS promoter activity was inhibited by isovitexin (Fig. 3D). The luciferase activity from LPS-activated cells was a 10.81-fold induction, which was suppressed to 3.47 fold by isovitexin. Therefore, isovitexin might block LPS-mediated inflammation via signaling.

Isovitexin decreased LPS-induced PGE_2 production and COX-2 expression in activated macrophages

Several studies in inflammation models have shown reciprocal interactions between NOS and cyclooxygenase pathways, most of which show stimulation of COX-2 by NO. Conversely, NOS enzymes can also be modulated by cyclooxygenase products. The concentration-response relationships were determined 18 h after treatment with LPS (50 ng/ml) alone or in the presence of isovitexin. Isovitexin showed reduction of PGE_2 generation in a concentration dependent manner with an IC_{50} about 80.0 μM (Fig. 4A). The inhibition was not due to general cellular toxicity (data not shown). COX-2 protein in activated cells was also suppressed by isovitexin in a concentration-dependent manner after 18 h treatment. The relative level of COX-2 protein with the treatment of 20, 40, 60, 80, and 100 μM isovitexin was 1.02, 0.82, 0.80, 0.71, and 0.58, repectively, versus LPS alone (Fig 4B). PGE_2 production and COX-2 protein level exhibited less interference by isovitexin than that of NO and iNOS in activated macrophages.

Suppression of LPS-induced NF-κB activation by isovitexin in RAW 264.7 cells

It has been documented that the transcription factor NF-κB is involved in the activation of iNOS by LPS induction. It needs to be asked whether isovitexin perturbs the translocation of NF-κB into the nucleus. Nuclear and cytosolic fractions of LPS-activated cells with time courses (0, 15, 30, 60 min, respectively) were prepared and subjected to immunoblot analysis. The amounts of nuclear p65 significantly increased during the 60-mintime course, during which the cytoplasmic fraction decreased (Fig. 5A). Co-incubation with LPS plus isovitexin resulted in the reduction of nuclear content of p65 protein, which was almost all retained in the cytoplasm. Thus, isovitexin is able to block LPS-mediated NF-κB nuclear translocation and activation. To further confirm the result, NF-κB promoter-drived luciferase reporter gene (pNFκB-Luc) was transiently expressed in activated cells. The luciferase activity from LPS-activated cells was a 5.51-fold elevation, which was suppressed to 2.84 fold by isovitexin (Fig. 5B). The LPS-induced NF-κB promoter activity was inhibited by isovitexin. Therefore, isovitexin blocks LPS-mediated inflammation via NF-κB signaling.

Inhibitory effects of isovitexin on LPS-induced degradation of IκBα

Since LPS-mediated activation of NF-κB results from the hyperphosphorylation of IκBα and its subsequent degradation, we examined the IκBα protein levels in activated macrophages. Cytosolic fraction of LPS-activated cells with time courses (0, 15, 30, 60 min, respectively) were prepared and subjected to immunoblot analysis. IκBα protein was detected by anti-IκBα antibody. Upon stimulation with LPS, the amounts of IκBα decreased during the 60-min time course, while isovitexin treatment resulted in maintaining fairly constant IκBα protein level (Fig. 5C). It can be suggested that inhibition of NO production by isovitexin occurs via blocking the phosphorylation, as well as degradation of IκBα protein, thus preventing the activation and translocation of NF-κB to the nucleus.

It is widely believed that oxidative DNA damage over the long human lifespan is a significant contributor to the development of the major cancers. Diet or chemopreventive agents that decrease oxidative DNA damage could delay or prevent the onset of cancer. ROS released by phagocytic cells are involved in the link between inflammation and cancer. Excessive and persistent formation of ROS by inflammatory cells is thought to be a key factor in the genotoxic effect. Different ROS affect DNA in different ways. It is believed that most of the H_2O_2-mediated DNA damage is due to the production of hydroxyl radical via the Fenton reaction [39]. Hydroxyl radical generates a multiplicity of products from all four DNA bases. The most commonly produced base lesion is 8-hydroxyguanine, which is often used as an index of oxidative DNA damage [40]. In addition, free radical-mediated reactions can cause multiple lesions and activate the nuclear enzyme poly(ADP-ribose) synthetase (PARS) (a protein modifying and nucleotide-polymerizing enzyme also known as PARP). PARS-related suicide pathway might play a role in various pathophysiological conditions in vivo [1]. In our study, isovitexin showed effective DNA-protection activity, i.e., effectively inhibiting pUC19 DNA strand break induced by the Fenton reaction. This compound, with the ability to protect DNA from the hydroxyl radical damage, may suppress oxidative DNA damage and/or the DNA damage-mediated signaling pathways, which involve various forms of cellular injury.

Persistent inflammation is a significant risk factor for several types of cancer. Inflammation can be a significant source of oxidative damage. Activated macrophages and neutrophils release a number of ROS, including H_2O_2, nitric oxide, superoxide, and hydroxyl radical, all of which can damage the DNA of nearby cells. H_2O_2 is produced by a variety of intracellular reactions upon stimuli [41]. The intracellular source of H_2O_2 is elevated by the activation of NAD(P)H oxidase, xanthine oxidase, and respiratory burst [42,43]. ROS was first proposed to be the common second messenger for activation of NF-κB by Schreck [44]. H_2O_2 has also been shown to activate NF-κB in Hela cells. In another study, H_2O_2 has been implicated in platelet-derived growth factor signal transduction involving p42/p44 mitogen-activated protein kinase, and JNK activation. The model was therefore proposed whereby diverse agents all activated NF-κB by causing oxidative stress [45]. Antioxidants, such as N-acetylcycteine, pyrrolidine dithiocarbamate, vitamin E, and vitamin C have been used to inhibit NF-κB activation in response to diverse stimuli [46]. The signaling pathway-mediated inflammation was proposed based on our studies, in which the LPS triggers protein kinase C activity, hydrogen peroxide production, MAP kinase activated IκB phosphorylation, and NF-κB translocation from cytoplasm into the nucleus, activating iNOS for NO accumulation and resulting in promotion of tumor progression by stimulating COX-2 expression [47]. High prostaglandin production by tumors results from upregulation of COX-2, which has been documented in several human cancers [48]. Chemotherapeutic or chemopreventic drugs able to inhibit both COX-2 and iNOS may become important adjuvants for inclusion in therapeutic protocols [2].

The effects of oxidants on signaling pathways are often characterized as resulting from oxidative stress. Our group reported that many antioxidants possessed anti-inflammatory effects. Theses findings provided a significant molecular basis for understanding the mode of actions of biochemically active dietary compounds in preventing cancer and inflammation. Figure 6 summarizes the roles of isovitexin during the process of carcinogenesis and inflammation and illustrates that isovitexin inhibited the LPS-induced NO and PGE_2 production in mouse macrophage RAW 264.7 cells (Fig. 3 and 4). Isovitexin displayed xanthine oxidase, an

intracellular enzymatic ROS source, inhibitory activity with blockade on its substrate-binding site [49]. PKC-activated xanthine oxidase activity upon LPS induction may be hampered by isovitexin which can reduce ROS generation in cells. Isovitexin possessed high antioxidant activity in previous studies [30], as well as in our own. Our findings demonstrate that isovitexin can block activation of NF-κB by interfering with the phosphorylation of IκB via its preventive effect on ROS generation and its ROS scavenger activity. Therefore, IκB still binds to NF-κB and prevents NF-κB translocation from the cytoplasm into the nucleus (Fig. 5A). Recently, activation of p38 or ERK-1 and -2 has been shown to be involved in stimulation of NF-κB activity and subsequent expression of iNOS and COX-2 in murine macrophages [50-52]. Protein tyrosine kinase has been implicated in NF-κB activation by LPS, UV light and hypoxia [53]. Isovitexin might also inhibit these kinase activities, inhibiting NF-κB activation upstream of the IκB phosphorylation. Immunoblot analysis supports that the inhibition of NF-κB activity by isovitexin might result from the inhibition of IκBα phosphorylation, degradation, and then reduction of the translocation of NF-κB subunits (Fig. 5A). However, isovitexin might also inhibit other signaling pathways induced by LPS which may be involved in the gene transcription of COX-2 and iNOS. Recently, our laboratories have also indicated that some flavonoids have the potential to inhibit the activity of IKK [54]. It remains to be elucidated if isovitexin correlates with suppression of IKK activity. Taken together, isovitexin is a food phytochemical in rice products that may play a role in the prevention of carcinogenesis and inflammation through suppressing NF-κB activation.

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