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A protective mechanism of licorice (*Glycyrrhiza uralensis*): Isoliquiritigenin stimulates detoxification system *via* Nrf2 activation

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ABSTRACT

Ethnopharmacological relevance: Licorice *(Glycyrrhizae radix)*, the root of *Glycyrrhiza uralensis* Fisch. (Leguminosae), is mainly used to moderate the characteristics of toxic herbs in Traditional Chinese Medicine, which could be partly interpreted as detoxification. However, the underlying mechanism is still not fully elucidated. Nuclear factor erythroid 2-related factor 2 (Nrf2) plays a key role in the protection against toxic xenobiotics. In our previous research, we have identified that extracts from *Glycyrrhiza uralensis* induced the expression of Nrf2 nuclear protein and its downstream genes. This research aims to screen the most potent Nrf2 inducer isolated from *Glycyrrhiza uralensis* and examine its effect on Nrf2 signaling pathway and detoxification system.
Materials and methods: Four compounds derived from *Glycyrrhiza uralensis* (glycyrrhetinic acid, liquiritigenin, isoliquiritigenin and liquiritin) were screened by ARE-luciferase reporter. The most potent ARE-luciferase inducer was chosen to further examine its effect on Nrf2 and detoxification genes in HepG2 cells. The role of Nrf2-dependent mechanism was tested by using Nrf2 knockout mice (Nrf2 KO) and Nrf2 wild-type mice (Nrf2 WT).
Results: ARE-luciferase reporter assay showed these four compounds were all potent Nrf2 inducers, and isoliquiritigenin was the most potent inducer. Isoliquiritigenin significantly up-regulated the expression of Nrf2 and its downstream detoxification genes UDP-glucuronosyltransferase 1A1 (UGT1A1), glutamate cysteine ligase (GCL), multidrug resistance protein 2 (MRP2) and bile salt export pump (BSEP) *in vitro* and *in vivo*. Additionally, isoliquiritigenin showed Nrf2-dependent transactivation of UGT1A1, GCLC and MRP2.
Conclusions: Isoliquiritigenin, isolated from *Glycyrrhiza uralensis*, stimulates detoxification system *via* Nrf2 activation, which could be a potential protective mechanism of licorice.

1. Introduction

Traditional Chinese Medicine (TCM) has focused on herb–herb interactions because most herbs are used together with others in a single prescription. Moreover, the combination therapies have been validated and show potential clinical benefits (Wang et al., 2013). Licorice (Glycyrrhizae Radix), the root of *Glycyrrhiza uralensis* Fisch. (Leguminosae), is one of the most popular herbal medicines, which appears in more than half of TCM prescriptions. According to TCM theory, it has a unique effect on moderating the characteristics of toxic herbs, which could be partly interpreted as detoxification (Qiao et al., 2012). The detoxification effect of licorice requires further investigations for the underlying mechanisms.

Induction of phase II drug metabolism enzymes and the efflux transporters promotes the detoxification and excretion of toxic substances, which is considered as one of the important approaches for drug detoxification (Zollner et al., 2010). Nrf2 plays a significant role in mediating phase II drug metabolism enzymes and the efflux transporters expression (Shen and Kong, 2009). Under physiological conditions, Nrf2 is present in the cytoplasm binding to the Kelch-like ECH-associated protein 1 (Keap1) which functions as a negative regulator of Nrf2 by preventing Nrf2 from entering into the nuclear and promoting its proteasomal degradation. Under stress conditions, Nrf2 dissociates from Keap1 and translocates into the nuclear

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where it dimerizes with small Maf protein and binds to antioxidant-responsive element (ARE), leading to expression of target genes (Ma and He, 2012). Additionally, a recent study has been reported that licorice induces the expression of Nrf2 mRNA (Wu et al., 2011). We hypothesized that activation of Nrf2 signaling pathway and its downstream genes could be one of licorice detoxification mechanisms.

In our previous study, we have identified that licorice extracts induced the expression of Nrf2 nuclear protein and its downstream genes (Gong et al., 2014). To further investigate the molecular mechanisms that licorice affects Nrf2 signaling pathway and its downstream detoxification genes, we screened the most potent ARE-luciferase inducer among four major compounds derived from *Glycyrrhiza uralensis*, namely glycyrrhetinic acid, liquiritigenin, isoliquiritigenin and liquiritin, and confirmed its effect on Nrf2 and its downstream genes, including phase II drug metabolism enzymes UGT1A1, GCLC and the efflux transporters MRP2, BSEP *in vitro* and *in vivo*.

2. Material and methods

2.1. Chemicals and reagents

Four major active components of licorice (glycyrrhetinic acid, liquiritigenin, isoliquiritigenin and liquiritin) were derived from the root of *Glycyrrhiza uralensis* Fisch. (Leguminosae). The plant materials were authenticated by Prof. Shao Liu (School of Pharmaceutical Sciences, Central South University, Changsha, China). The voucher specimens were deposited in the Herbarium, School of Pharmaceutical Sciences, Central South University, Changsha, China. The work of extraction, separation and refining was finished in OnRoad Biotechnology Co., Ltd. (Changsha, China). Deserved to be mentioned, Glycyrrhetinic acid was prepared by acid hydrolysis of glycyrrhizic acid extracted from *Glycyrrhiza uralensis*. The purity of glycyrrhetinic acid, liquiritigenin, isoliquiritigenin and liquiritin were 99.27%, 99.45%, 99.85% and 99.89%, respectively. Structures of them were listed in Fig. 1. Tert-butyl hydroquinone (tBHQ), 3-(4, 5-dimethyl-thiazol-2-yl) 2, 5-diphenyl tetrazolium bromide (MTT) and dimethylsulfoxide were purchased from Sigma-Aldrich (St. Louis, Mo). Anti-Nrf2 and anti-Lamin B antibodies were purchased from Santa Cruz (Santa Cruz, CA). Other chemicals were of analytical grade from commercial suppliers.

2.2. Cell culture

Human hepatocarcinoma (HepG2) cells obtained from Chinese Academy of Sciences Cell Bank (Shanghai, China) were cultured in Minima Essential Medium (MEM) (Hyclone, USA) supplemented with 10% fetal bovine serum (Hyclone, USA) in a 37 °C incubator with 5% CO_2.

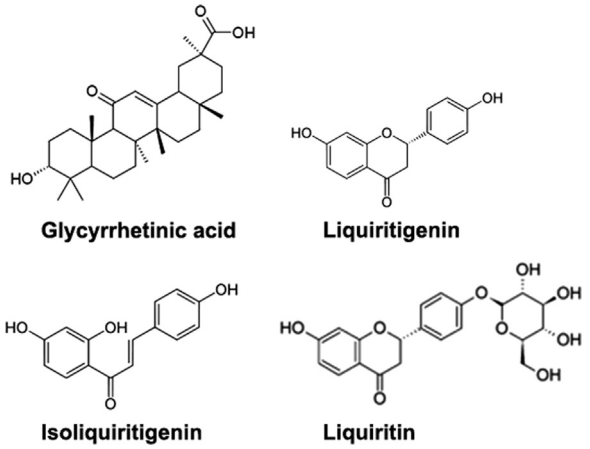

Fig. 1. Chemical structures of four compounds (glycyrrhetinic acid, liquiritigenin, isoliquiritigenin and liquiritin) derived from *Glycyrrhiza uralensis*.

2.3. MTT assay

HepG2 cells (1×10^4) were seeded in 96-well plates. After incubation for 24 h, the cells were treated with control (0.1% DMSO), glycyrrhetinic acid, liquiritigenin, isoliquiritigenin, or liquiritin. The final concentration of these test extracts was 10–50 μM. Cell viability was determined by a rapid colorimetric assay using MTT as described in the manufacturer's instructions. The results were obtained from three independently conducted experiments.

2.4. ARE-luciferase reporter assay

The Dual-Luciferase Reporter Assay System (Promega, UK) was used to determine the reporter gene activity in transiently transfected cells. Transient transfection was performed in 96-well plates at a cell density of 50–70% confluence per well. Then the 8 × ARE pGL3 plasmid was co-transfected with the pRL-TK plasmid (transfection ratio of 8 × ARE pGL3: pRL-TK is 10:1), encoding *Renilla luciferase* as an internal control for transfection efficiency for 24 h using Lipofectamine 2000 (Invitrogen) according to the manufacturer's instructions. After transfection, cells were treated with test samples for 8 h, and then cell lysates were prepared for assessment of luciferase activity. Fire fly and *Renilla luciferase* activities were measured by a luminometer (Centro XS3 LB960, Berthold, German) according to the manufacturer's instructions. Relative fire fly luciferase activity was normalized to *Renilla luciferase* activity and activity was expressed as fold induction after treatment with compounds compared with the control.

2.5. Western blot analysis

Cell extracts were prepared in RIPA buffer (Beyotime, China). Nuclear extracts were prepared with a Nuclear Extract Kit (Thermo, USA) according to the manufacturer's recommendations. Equivalent amounts of protein were separated by 12% SDS-polyacrylamide gel electrophoresis and transferred to polyvinylidene difluoride membranes. After being blocked in 5% non-fat milk in TBST for 1 h at room temperature, the membranes were incubated with the primary antibodies anti-Nrf2 (1:500) and lamin B (1:1000) or GAPDH (1:1000) at 4 °C overnight. The immunoblots were then incubated with a secondary antibody for 2 h at room temperature. The membranes were developed with an electrochemiluminescence (ECL) kit (Thermo) according to the manufacturer's protocol.

2.6. Quantitative real-time PCR analysis

Total mRNA was extracted from the cells or the liver with TRIZOL reagent (Invitrogen) and equal amounts of RNA were reversely-transcripted to cDNA with Maxima® First Strand cDNA Synthesis Kit (Fermentas, Canada). The cDNA was analyzed by Quantitative real-time PCR with Maxima® SYBR Green/ROX qPCR Master Mix (Fermentas). The level of GAPDH mRNA was used as an internal standard. The primers for real-time PCR analysis are shown in Tables 1 and 2.

2.7. Animals and in vivo study

Six to eight week old female ICR Nrf2 KO mice and ICR WT mice were purchased from The Jackson Laboratory (USA) and housed in a climate-controlled, circadian rhythm-adjusted room and allowed food and water *ad libitum*. Six mice were used in each group. The mice were treated with control (0.5% CMC-Na) or isoliquiritigenin

Table 1
Murine primers for q-PCR.

Gene	Forward	Reverse
GAPDH	5′-CTC CCA CTC TTC CAC CTT CG-3′	5′-CCA CCA CCC TGT TGC TGT AG-3′
UGT1A1	5′-GGA GGC TGT TAG TGT TCC CT-3′	5′-CCG TCC AAG TTC CAC CAA AG-3′
GCLC	5′-GGC CAC TAT CTG CCC AAT TG-3′	5′-TGT TCT TCA GAG GCT CCA GG-3′
MRP2	5′-ATG GGA CCG ACA ATT CAC CT-3′	5′-CCC GGC AAA TCT GTT CAC AA-3′
BSEP	5′-CAT CGC TGG GTT TGA GGA TG-3′	5′-CAG CAG CCT TTT CGT CAC TT-3′

Table 2
Human primers for q-PCR.

Gene	Forward	Reverse
GAPDH	5′-TGC ACC ACC AAC TGC TTA GC-3′	5′-GGC ATG GAC TGT GGT CAT GAG-3′
UGT1A1	5′-TAA GTG GCT ACC CCA AAA CG-3′	5′-TCC AGC TCC CTT AGT CTC CA-3′
GCLC	5′-CTG TTG CAG GAA GGC ATT GAT-3′	5′-TTC AAA CAG TGT CAG TGG GTC TCT-3′
MRP2	5′-AAG AGT CTT CGT TCC AGA CGC-3′	5′-GGG AAT CCA CAC CAG AAC AGT-3′
BSEP	5′-AGG GAA ATC AAG CTC TTA ATG AAG-3′	5′-ATA GGT AGA CTT ATG ATC TAC AAC A-3′

25 mg/kg by oral gavage. After dosing for three days, the livers were collected for q-PCR and Western blot. All procedures were carried out in accordance with the Regulations of Experimental Animal Administration issued by the State Committee of Science and Technology of the People's Republic of China, with the approval of the Ethics Committee in Jiangsu Institute of Chinese Medicine.

2.8. Statistical analysis

Results from the experiment were reported as means ± S.D. and analyzed with SPSS 19.0. Differences between the groups were determined by one-way ANOVA test followed by Tukey's test. The prior level of significance was established at $p < 0.05$ or $p < 0.01$.

3. Results

3.1. Effects of the four compounds on ARE luciferase activity

To determine whether ARE-mediated transcriptional activities are affected by these four compounds, HepG2 cells were transfected with pARE-luciferase reporter plasmid and treated with glycyrrhetinic acid, liquiritigenin, isoliquiritigenin and liquiritin with various concentrations for 8 h. tBHQ was used as a positive control. Cell viability was measured by MTT assay (Fig. 2). As shown in Fig. 3, Isoliquiritigenin was the most potent ARE-luciferase inducer among the four compounds.

3.2. Effects of isoliquiritigenin on the activation of Nrf2

To further determine whether isoliquiritigenin affects the level of the Nrf2 protein, HepG2 cells were treated with isoliquiritigenin for 8 h and the level of Nrf2 protein was determined by Western blot analysis. As shown in Fig. 4, significant induction of Nrf2 by isoliquiritigenin was observed at 5 μM, then further enhanced at the concentration range of 10–20 μM, and declined at 25 μM.

Since Nrf2 nuclear translocation is a key event in the activation of this pathway, we further examined Nrf2 nuclear accumulation after isoliquiritigenin treatment. As shown in Fig. 5, treatment with isoliquiritigenin (20 μM) for 8 h significantly increased the nuclear levels of Nrf2. tBHQ was utilized as a positive control.

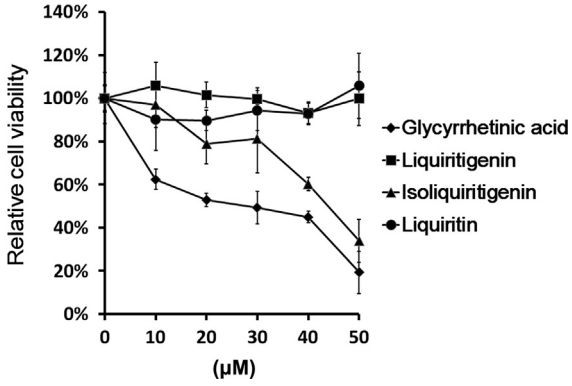

Fig. 2. Cytotoxicity of the four compounds. HepG2 cells were treated with glycyrrhetinic acid, liquiritigenin, isoliquiritigenin and liquiritin at various concentrations for 24 h. Cell viability was determined by an MTT assay. The data are represented as mean ± S.D. from three independent experiments.

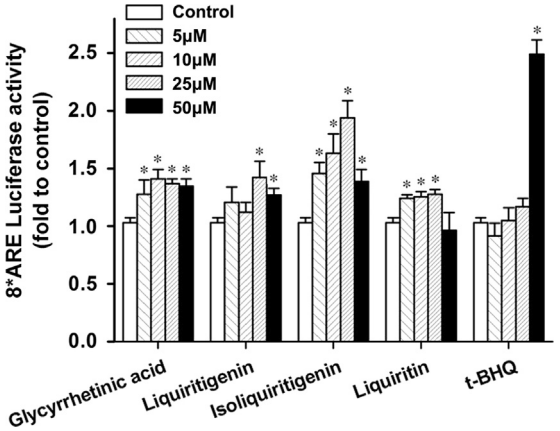

Fig. 3. Effects of the four compounds on ARE-reporter activity. HepG2 cells were transfected with a pGL3 plasmid containing the ARE-motif. After transfection, cells were treated with test samples for 8 h. Data are expressed as fold of induction of luciferase activity compared to the control (mean ± S.D.;$n = 3$). *$P < 0.05$ *versus* the control.

3.3. Effects of isoliquiritigenin on the induction of detoxification genes

To examine whether isoliquiritigenin has stimulating effect on detoxification genes, qPCR analysis was performed. As shown in

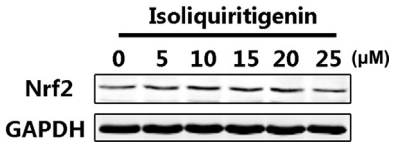

Fig. 4. Effects of isoliquiritigenin on the levels of Nrf2 protein. After being exposed to the indicated concentrations of isoliquiritigenin (5, 10, 15, 20, 25 μM) for 8 h, the total protein was prepared and subjected to Western blot using the indicated antibodies. The GAPDH was used as an internal control.

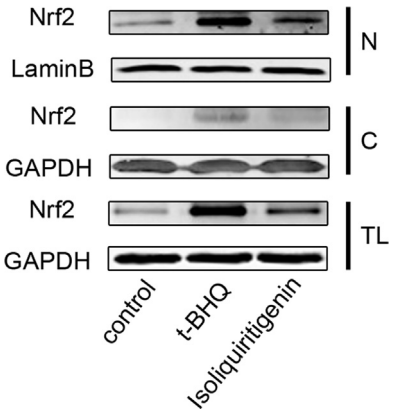

Fig. 5. Effects of isoliquiritigenin on the nuclear levels of Nrf2 protein. After being exposed to isoliquiritigenin (20 μM) for 8 h, the total protein (TL), cytoplasmic protein (C) and nuclear protein (N) were prepared and subjected to Western blot analysis using the indicated antibodies. The GAPDH and lamin B were used as the internal control. tBHQ (25 μM) was used as the positive control.

Fig. 6, the expression of UGT1A1, GCLC, MRP2 and BSEP was induced by isoliquiritigenin in a dose-dependent manner.

3.4. *Effects of isoliquiritigenin on the expression of Nrf2 and detoxification genes in the liver of Nrf2 WT and Nrf2 KO mice*

As shown in Fig. 7, the nuclear levels of Nrf2 were increased in the livers of WT mice, which was consistent with the results of cell experiments. To further investigate if the activation of relevant detoxification genes caused by isoliquiritigenin was mediated *via* Nrf2 signaling pathway, the expression levels of these genes in the liver of Nrf2 KO and WT mice treated with isoliquiritigenin were measured by qPCR. As shown in Fig. 8, UGT1A1, GCLC, MRP2 and BSEP mRNA were substantially induced in WT mice, but this induction was significantly attenuated in Nrf2 KO mice except BSEP. However, the basal levels of UGT1A1, MRP2 and BSEP were increased in Nrf2 KO mice control compared with the WT mice control.

4. Discussion

Licorice has been reported to have multiple pharmacological effects, such as anti-cancer, anti-inflammatory and hepatoprotective effects (Asl and Hosseinzadeh, 2008), but it is more commonly used to moderate the characteristics of toxic herbs in traditional and modern formulas. However, the precise cellular and molecular mechanisms remain unclear. In addition, the Nrf2 pathway is presumably the most important transcriptional factor for the cell to deal with oxidative stress and toxicity generated from exposure to exogenous and endogenous chemicals (Niture et al., 2010). Studies have revealed that Nrf2 knockout mice were more susceptible to butylated hydroxyltoluene induced lung injury and

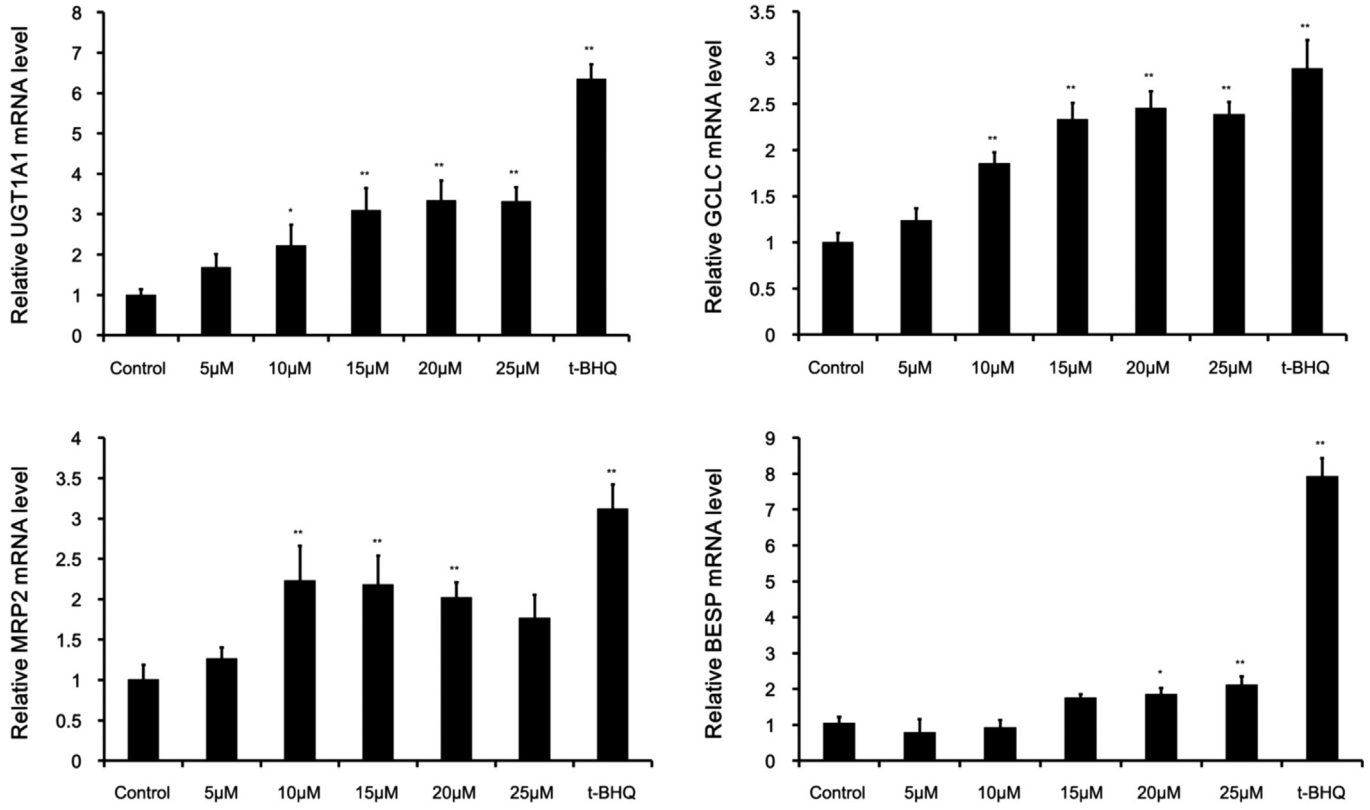

Fig. 6. Effects of isoliquiritigenin on mRNA expression of UGT1A1, GCLC, MRP2 and BSEP genes in cells. After being exposed to the indicated concentrations of isoliquiritigenin (5, 10, 15, 20, 25 μM) for 8 h, the mRNA expressions of UGT1A1 (A), GCLC (B), MRP2 (C) and BSEP (D) were analyzed by Quantitative real-time PCR. The GAPDH was used as an internal control. tBHQ (25 μM) was used as the positive control. The data are represented as mean ± S.D. from three independent experiments. $^{*}P < 0.05$, $^{**}P < 0.01$ *versus* the control.

acetaminophen induced hepatotoxicity at high dose (Chan and Kan, 1999; Enomoto et al., 2001). Since the effect of licorice extracts on Nrf2 and its downstream genes has been confirmed, we screened the most potent ARE-luciferase inducer, considering that the effect of monomer is much clearer, which will help us understand the role of Nrf2 in licorice detoxification more accurately. Glycyrrhizic acid, the main ingredient in licorice, is metabolized into glycyrrhetinic acid which is eventually absorbed into the blood and exerts its bioactivity in human body. Liquiritigenin, isoliquiritigenin and liquiritin are the most representative of glycyrrhiza flavonoids. In our study, glycyrrhetinic acid, liquiritigenin, isoliquiritigenin and liquiritin have been chosen as they are the main active constituents in licorice (Asl and Hosseinzadeh, 2008; Ohno et al., 2013), and they were screened with ARE luciferase reporter. The results showed isoliquiritigenin was the most potent ARE luciferase inducer (Fig. 3).

Isoliquiritigenin has been evaluated in terms of its anti-oxidative effects (Chin et al., 2007), anti-allergic effects (Kumar et al., 2007) and anti-tumor effects (Zhao et al., 2013). Furthermore, a recent study has indicated isoliquiritigenin facilitates differentiation in human promyelocytic leukemia cells (HL-60), and the mechanisms should be involved in Nrf2/ARE pathway (Chen et al., 2013). In the present study, isoliquiritigenin markedly activated the Nrf2/ARE pathway, as reflected by increased Nrf2 protein level and increased nuclear accumulation of Nrf2 (Figs. 4 and 5).

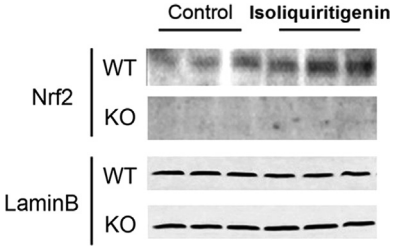

Fig. 7. Effects of isoliquiritigenin on the nuclear levels of Nrf2 protein in mice. Mice in all groups were treated as the same with the above method. The expression of Nrf2 and lamin B in the liver was determined by Western blot. The lamin B was used as an internal control.

Activation of Nrf2 signal pathway plays pivotal roles in preventing xenobiotic-related toxicity (Shen et al., 2005). Previous studies showed Nrf2 protected against toxin-induced liver injury through transcriptional up-regulation of an array of downstream genes, such as GCL, HO-1, GST, NQO1 and MRPs (Jiang et al., 2009; Reisman et al., 2009), suggesting that the protective role of Nrf2 has been attributed partly to its involvement in coordinated induction of phase II drug metabolism enzymes and Phase III drug transporters (Klaassen and Slitt, 2005). UGT1A1 functions to detoxify by catalyzing the glucuronidation of structurally diverse compounds, making them more water-soluble and more easily excreted into bile and urine by transporters (Yueh and Tukey, 2007). GCLC is the catalytic subunit of GCL, the enzyme that catalyzes the rate-limiting step in the biosynthesis of GSH (Baird and Dinkova-Kostova, 2011), which could combine with the toxic components, leading to excretion. MRP2 participates in excretion of chemicals into bile, especially glutathione-, glucuronide- and sulfate-conjugated metabolites (Vollrath et al., 2006). BSEP is the major determinant of bile salt-dependent bile secretion, deficiencies of which always lead to progressive cholestatic liver injury (Weerachayaphorn et al., 2009). Our results showed that isoliquiritigenin significantly induced UGT1A1, GCLC, MRP2 and BSEP mRNA expression (Fig. 6). The activation of these genes conduces to decrease the oxidative burden of the cell by increasing GSH concentrations and general detoxification of chemicals *via* phase II conjugation reactions as well as efflux transportation.

To further investigate if the effect of isoliquiritigenin is Nrf2-dependent, Nrf2 WT and Nrf2 KO mice were gavaged with isoliquiritigenin. The inducible expression of UGT1A1, GCLC and MRP2 by isoliquiritigenin was attenuated in Nrf2 KO mice,

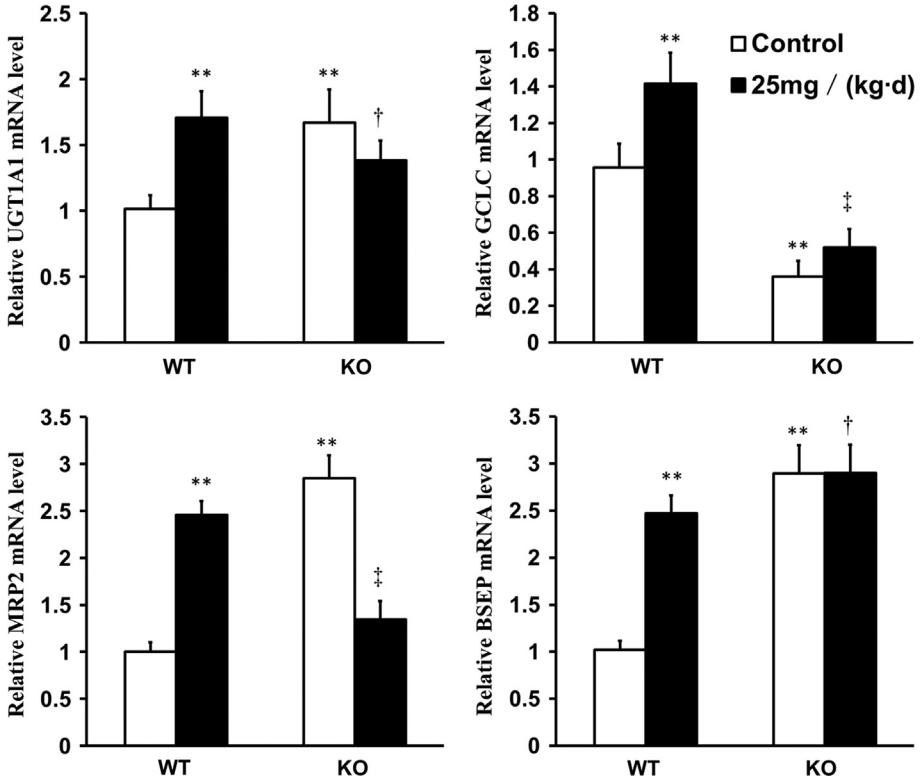

Fig. 8. Effects of isoliquiritigenin on mRNA expression of UGT1A1, GCLC, MRP2 and BSEP genes in mice. Mice in all groups were treated as the same with the above method. The mRNA expression levels of UGT1A1 (A), GCLC (B), MRP2 (C) and BSEP (D) genes in the liver were determined by Quantitative real-time PCR. The data are represented as mean ± S.D. $^{*}P < 0.05$, $^{**}P < 0.01$ *versus* Nrf2 WT control. †$P < 0.05$, ‡$P < 0.01$ *versus* Nrf2 WT isoliquiritigenin.

indicating that the UGT1A1-, GCLC- and MRP2-inducible expression are Nrf2-dependent (Fig. 8). Nrf2 is important not only for the induction of GCLC but also for its constitutive level. In Nrf2 KO mice, the constitutive expression of GCLC mRNA is approximately 62% lower than in WT mice. Interestingly, the constitutive expression of UGT1A1, MRP2 and BSEP had been up-regulated in Nrf2 KO mice. This phenomenon could be explained by bile acid homeostasis. A possible biological adaptation event to reduce the bile acid following Nrf2 gene knockout has been reported (Aleksunes et al., 2006). The expression of farnesoid X receptor (FXR) is increased in Nrf2 KO mice (Weerachayaphorn et al., 2012), enhancing the bile acid excretion *via* up-regulation of BSEP. Elevations in hepatic pregnane X receptor (PXR) in Nrf2 KO mice are associated with increased production of phase I enzyme for bile acid hydroxylation reactions. Then bile acids undergo phase II reactions by sulfation and glucuronidation conjugation reactions. Moreover, the key enzyme for sulfo-conjugation of bile acids (Sulfotransferase 2A1) is Nrf2-dependent (Alnouti and Klaassen, 2008). Therefore, the increase of UGT1A1 might help compensate for the diminished sulfation reactions in the Nrf2 KO mice. Clear regulation of downstream target genes is not uniform (Maher et al., 2007). MRP2 are also regulated by FXR and PXR (Zollner et al., 2010), which might lead to the increase of MRP2 mRNA in the Nrf2 KO mice. These multiple adaptive changes could be the reason why UGT1A1, MRP2 and BSEP were induced in Nrf2 KO mice. Even though these genes could be regulated by multiple factors, the effect of isoliquiritigenin on the induction of UGT1A1, GCLC and MRP2 is Nrf2-dependent.

In summary, isoliquiritigenin isolated from licorice significantly induces Nrf2 and its downstream detoxification genes GCLC, UGT1A1, MRP2 and BSEP. In addition, isoliquiritigenin induces GCLC, UGT1A1 and MRP2 in Nrf2 WT mice but less so in Nrf2 KO mice, suggesting that the inducible expression of these genes is Nrf2-dependent. Notably, these data may represent, at least in part, a novel mechanism for the traditional use of licorice to alleviate the side effects of toxic traditional Chinese medicines.

Acknowledgments

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References

Aleksunes, L.M., Slitt, A.L., Maher, J.M., Dieter, M.Z., Knight, T.R., Goedken, M., Cherrington, N.J., Chan, J.Y., Klaassen, C.D., Manautou, J.E., 2006. Nuclear factor-E2-related factor 2 expression in liver is critical for induction of NAD(P)H: quinone oxidoreductase 1 during cholestasis. Cell Stress Chaperones 11, 356–363.

Alnouti, Y., Klaassen, C.D., 2008. Regulation of sulfotransferase enzymes by prototypical microsomal enzyme inducers in mice. Journal of Pharmacology and Experimental Therapeutics 324, 612–621.

Asl, M.N., Hosseinzadeh, H., 2008. Review of pharmacological effects of Glycyrrhiza sp. and its bioactive compounds. Phytotherapy Research 22, 709–724.

Baird, L., Dinkova-Kostova, A.T., 2011. The cytoprotective role of the Keap1-Nrf2 pathway. Archives of Toxicology 85, 241–272.

Chan, K., Kan, Y.W., 1999. Nrf2 is essential for protection against acute pulmonary injury in mice. Proceedings of the National Acadamy of Science USA 96, 12731–12736.

Chen, H., Zhang, B., Yuan, X., Yao, Y., Zhao, H., Sun, X., Zheng, Q., 2013. Isoliquiritigenin-induced effects on Nrf2 mediated antioxidant defence in the HL-60 cell monocytic differentiation. Cell Biology International 37, 1215–1224.

Chin, Y.W., Jung, H.A., Liu, Y., Su, B.N., Castoro, J.A., Keller, W.J., Pereira, M.A., Kinghorn, A.D., 2007. Anti-oxidant constituents of the roots and stolons of licorice (*Glycyrrhiza glabra*). Journal of Agricultural and Food Chemistry 55, 4691–4697.

Enomoto, A., Itoh, K., Nagayoshi, E., Haruta, J., Kimura, T., O'Connor, T., Harada, T., Yamamoto, M., 2001. High sensitivity of Nrf2 knockout mice to acetaminophen hepatotoxicity associated with decreased expression of ARE-regulated drug metabolizing enzymes and antioxidant genes. Toxicological Sciences 59, 169–177.

Hui Gong, Huan-de Li, Yan, Miao, Zhang, Bi-kui, Jiang, Pei, Fan, Xin-rong, Deng, Yang, 2014. Effect of licorice on the induction of phase II metabolizing enzymes and phase III transporters and its possible mechanism. Pharmazie, http://dx.doi.org/10.1691/ph.2014.4615.

Jiang, T., Huang, Z., Chan, J.Y., Zhang, D.D., 2009. Nrf2 protects against As(III)-induced damage in mouse liver and bladder. Toxicology and Applied Pharmacology 240, 8–14.

Klaassen, C.D., Slitt, A.L., 2005. Regulation of hepatic transporters by xenobiotic receptors. Current Drug Metabolism 6, 309–328.

Kumar, S., Sharma, A., Madan, B., Singhal, V., Ghosh, B., 2007. Isoliquiritigenin inhibits IkappaB kinase activity and ROS generation to block TNF-alpha induced expression of cell adhesion molecules on human endothelial cells. Biochemical Pharmacology 73, 1602–1612.

Ma, Q., He, X., 2012. Molecular basis of electrophilic and oxidative defense: promises and perils of Nrf2. Pharmacological Reviews 64, 1055–1081.

Maher, J.M., Dieter, M.Z., Aleksunes, L.M., Slitt, A.L., Guo, G., Tanaka, Y., Scheffer, G.L., Chan, J.Y., Manautou, J.E., Chen, Y., Dalton, T.P., Yamamoto, M., Klaassen, C.D., 2007. Oxidative and electrophilic stress induces multidrug resistance-associated protein transporters *via* the nuclear factor-E2-related factor-2 transcriptional pathway. Hepatology 46, 1597–1610.

Niture, S.K., Kaspar, J.W., Shen, J., Jaiswal, A.K., 2010. Nrf2 signaling and cell survival. Toxicology and Applied Pharmacology 244, 37–42.

Ohno, H., Araho, D., Uesawa, Y., Kagaya, H., Ishihara, M., Sakagami, H., Yamamoto, M., 2013. Evaluation of cytotoxiciy and tumor-specificity of licorice flavonoids based on chemical structure. Anticancer Research 33, 3061–3068.

Qiao, X., Ye, M., Xiang, C., Bo, T., Yang, W.Z., Liu, C.F., Miao, W.J., Guo, D.A., 2012. Metabolic regulatory effects of licorice: a bile acid metabonomic study by liquid chromatography coupled with tandem mass spectrometry. Steroids 77, 745–755.

Reisman, S.A., Aleksunes, L.M., Klaassen, C.D., 2009. Oleanolic acid activates Nrf2 and protects from acetaminophen hepatotoxicity *via* Nrf2-dependent and Nrf2-independent processes. Biochemical Pharmacology 77, 1273–1282.

Shen, G., Kong, A.N., 2009. Nrf2 plays an important role in coordinated regulation of Phase II drug metabolism enzymes and Phase III drug transporters. Biopharmaceutics and Drug Disposition 30, 345–355.

Shen, G., Jeong, W.S., Hu, R., Kong, A.N., 2005. Regulation of Nrf2, NF-kappaB, and AP-1 signaling pathways by chemopreventive agents. Antioxidants and Redox Signal 7, 1648–1663.

Vollrath, V., Wielandt, A.M., Iruretagoyena, M., Chianale, J., 2006. Role of Nrf2 in the regulation of the Mrp2 (ABCC2) gene. Biochemical Journal 395, 599–609.

Wang, X., Zhang, H., Chen, L., Shan, L., Fan, G., Gao, X., 2013. Liquorice, a unique "guide drug" of traditional Chinese medicine: a review of its role in drug interactions. Journal of Ethnopharmacology 150, 781–790.

Weerachayaphorn, J., Cai, S.Y., Soroka, C.J., Boyer, J.L., 2009. Nuclear factor erythroid 2-related factor 2 is a positive regulator of human bile salt export pump expression. Hepatology 50, 1588–1596.

Weerachayaphorn, J., Mennone, A., Soroka, C.J., Harry, K., Hagey, L.R., Kensler, T.W., Boyer, J.L., 2012. Nuclear factor-E2-related factor 2 is a major determinant of bile acid homeostasis in the liver and intestine. Amerian Journal of Physiology – Gastrointestinal and Liver Physiology 302, G925–G936.

Wu, T.Y., Khor, T.O., Saw, C.L., Loh, S.C., Chen, A.I., Lim, S.S., Park, J.H., Cai, L., Kong, A.N., 2011. Anti-inflammatory/anti-oxidative stress activities and differential regulation of Nrf2-mediated genes by non-polar fractions of tea *Chrysanthemum zawadskii* and licorice *Glycyrrhiza uralensis*. AAPS Journal 13, 1–13.

Yueh, M.F., Tukey, R.H., 2007. Nrf2-Keap1 signaling pathway regulates human UGT1A1 expression in vitro and in transgenic UGT1 mice. Journal of Biological Chemistry 282, 8749–8758.

Zhao, H., Yuan, X., Li, D., Chen, H., Jiang, J., Wang, Z., Sun, X., Zheng, Q., 2013. Isoliquiritigen enhances the antitumour activity and decreases the genotoxic effect of cyclophosphamide. Molecules 18, 8786–8798.

Zollner, G., Wagner, M., Trauner, M., 2010. Nuclear receptors as drug targets in cholestasis and drug-induced hepatotoxicity. Pharmacology and Therapeutics 126, 228–243.