Vol. 13, No. 4, 2022
ISSN 2233-4203/ e-ISSN 2093-8950
ARTICLE www.msletters.org | Mass Spectrometry Letters

Inhibitory Effects of Dietary Schisandra Supplements on CYP3A Activity in Human Liver Microsomes

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Received November 9, 2022, Revised December 13, 2022, Accepted December 21, 2022
First published on the web December 31, 2022; DOI: 10.5478/MSL.2022.13.4.152

Abstract : *Schisandra chinensis* and its fruits have been used as a traditional herbal medicine to treat liver dysfunction, fatigue, and chronic coughs. Several in vitro and in vivo studies suggested that dibenzocyclooctadiene lignans present in Schisandra fruits strongly inhibit CYP3A4 activity. However, reports on the inhibitory potential of dietary Schisandra supplements against CYP3A activity are limited despite their increasing consumption as dietary supplements. In this study, we evaluated the CYP3A-inhibitory potential of four dietary Schisandra supplements in human liver microsomes. At a concentration of 0.05 mg/mL, Schisandra supplements from Nature's Way, Swanson, Planetary Herbals, and Only Natural inhibited CYP3A activity by 93.9, 70.8, 33.6, and 24.8%, respectively. Nature's Way, which exhibited the strongest inhibition against CYP3A, had the highest contents of gomisin B and gomisin C, which potently inhibit CYP3A activity. The in vivo pharmacokinetics of this product should be examined to determine whether the clinical relevance of inhibiting CYP3A activity by dietary Schisandra supplementation.

Keywords : CYP3A, Inhibition, Lignan, Schisandra supplements

Introduction

The concomitant administration of dietary botanical supplements and prescribed drugs has increased worldwide. For example, approximately 18–20% of adults in the United States consume prescribed drugs concurrently with dietary supplements.[1] The concomitant use of dietary supplements and drugs may result in pharmacokinetic herbal medicine-drug interactions (HMDI) causing increased toxicity or decreased efficacy.[2]

Several medicinal herbs reportedly cause HMDI, including echinacea, ginseng, milk thistle, and St John's wort.[3] As a well- known example, consumption of St John's wort reduces the oral bioavailability of co-administered drugs, including cyclosporine, tacrolimus, and simvastatin by inducing CYP3A enzymes, eventually leading to insufficient drug effects.[4] Co-administration with grapefruit juice also increases the oral bioavailability of drugs, such as calcium channel blockers and HMG-CoA reductase inhibitors, which function as CYP3A substrates and inhibit CYP3A enzymes.[5]

Cytochrome P450 (P450) 3A enzymes are considered the most important human P450 owing to their high relative abundance in the liver and intestine and their involvement in the metabolism of over 50% of marketed drugs.[6] Therefore, evaluating the inhibitory effects of drug candidates or traditional herbal medicines against CYP3A enzymes is essential for developing new drugs. *Schisandra chinensis* Bailon and its fruits, known as omija, wuweizi, and gomishi in Korea, China, and Japan, respectively, have been used in herbal medicine to treat liver dysfunction, chronic coughs, and fatigue.[7] Several *in vitro* studies have suggested that dibenzocyclooctadiene lignans present in omija strongly inhibit CYP3A4 activity in a time-dependent manner.[8,9] A recent *in vivo* study also suggested that gomisin A, a type of omija lignan, participates in the pharmacokinetic interaction of cyclophosphamide by blocking CYP3A-mediated bioactivation, thus reducing chloroacetaldehyde production and playing a role in the chemopreventive activity of omija against cyclophosphamide toxicity.[10]

Owing to various pharmacological activities of Schisandra, its market is expected to grow with a compound annual growth rate (CAGR) of 7.56% during 2022-2029 (https://www.datamintelligence.com/research-report/schisandra-market). To date, reports on the inhibitory effect of dietary Schisandra supplements on CYP3A activity are limited.

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Therefore, we evaluate the potential of four representative dietary Schisandra supplements to inhibit CYP3A-mediated midazolam 1'-hydroxylase activity in human liver microsomes (HLMs). We also compare the difference in the CYP3A inhibitory potential of dietary Schisandra supplements from different manufacturers by analysing the omija lignan content.

Experimental

Materials

Gomisin A (≥98%), gomisin C (>98%), schisandrin (98%), deoxyschisandrin (≥98%), and wuweizisu C (≥98%) (Figure 1) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Gomisin B (95%) (Figure 1) and midazolam were purchased from Toronto Research Chemicals (Toronto, ON, Canada). Gomisin N (>99%) (Figure 1) was obtained from CoreSciences (Seoul, Korea). Pooled HLMs (XTreme 200, H2630, mixed gender) were supplied by XenoTech (Lenexa, KS, USA). All the other solvents used were of LC-MS grade (Fisher Scientific Co., Pittsburgh, PA, USA).

Samples of dietary Schisandra supplements

Dietary Schisandra supplements were obtained from local commercial sources or online shopping malls. Available information is presented in Table 1. Samples were stored at 4°C until further use. Dietary Schisandra supplement stock solutions (2.5 mg/mL) were prepared in 50% methanol.

Inhibitory effects of dietary Schisandra supplements against CYP 3A enzyme

The inhibitory potential of dietary *Schisandra* supplements on CYP3A activity was evaluated using our previously developed method, with slight modifications.[9] Briefly, microsomal incubation mixtures containing 0.1 M phosphate buffer (pH 7.4), pooled HLMs (0.25 mg/mL), midazolam as a CYP3A probe substrate (0.1 mM), and dietary Schisandra supplement samples (0.05 mg/mL) were pre-incubated (37°C, 5 min). Subsequently, the NADPH generating system was added and further incubated for 10 min. After quenching the incubated samples with cold acetonitrile, the samples were centrifuged. Aliquots of the supernatants were analyzed using liquid chromatography-tandem mass spectrometry (LC-MS/MS).[9]

LC-MS/MS analysis

1'-Hydroxymidazolam was separated on a Kinetex XB-C18 column (100 × 2.1 mm, 2.6 μm, Phenomenex, Torrance, CA, USA) and analyzed using a Shimadzu LC-MS 8060 system (Shimadzu, Kyoto, Japan). The mobile phase consisted of water containing 0.1% formic acid (A) and acetonitrile containing 0.1% formic acid (B). The elution conditions for the analysis of 1'-hydroxymidazolam were set as 8% B for 0–0.5 min, 8%→60% B for 0.5–5 min, 60% B for 5–6 min, 60%→8% B for 6–6.1 min, and 8% B for 6.1–9 min.[9] The mass transition and collision energy (CE) used for the quantitation of 1'-hydroxymidazolam were *m/z* 342 → 203 and 28 eV, respectively.

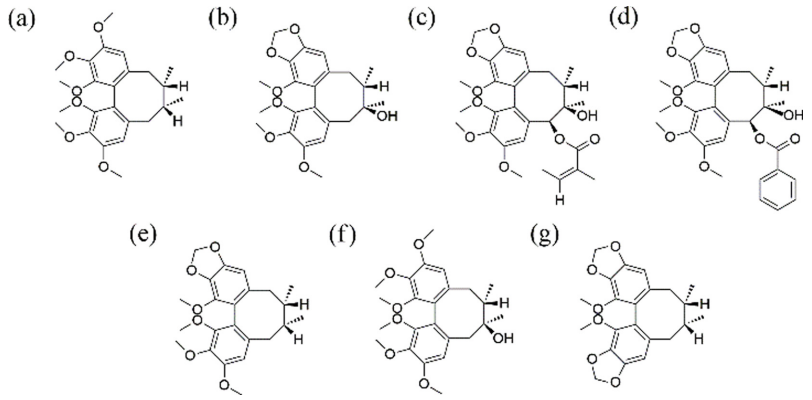

Figure 1. Chemical structures of the seven schisandra lignans: (a) deoxyschisandrin; (b) gomisin A; (c) gomisin B; (d) gomisin C; (e) gomisin N; (f) schisandrin; and (g) wuweizisu C.

Table 1. Available information about the commercial dietary Schisandra supplements used in this study.

Maker	Trade name	Herbal supplement	Amount per serving
Nature's Way	Schisandra Fruit	Schisandra	1,160 mg
Only Natural	Schizandra Extract	Schisandra extract	500 mg
Planetary Herbals	Schisandra Adrenal Complex	Schisandra fruit	1,420 mg
Swanson	Schizandra Berries	Schisandra berry	525 mg

Quantitative analysis of dibenzocyclooctadiene lignans in dietary Schisandra supplements

Dibenzocyclooctadiene lignans in dietary Schisandra supplements were analyzed using a LC-MS/MS method developed by our group.[11] Seven lignans were separated on a Kinetex C18 column (100 × 2.1 mm, 2.6 μm). The mass transitions used for quantitation of gomisin A, gomisin B, gomisin C, gomisin N, deoxyschisandrin, wuweizisu C, and schisandrin were *m/z* 417 → 399 (CE 20 eV), *m/z* 537 → 415 (CE 25 eV), *m/z* 554 → 415 (CE 20 eV), *m/z* 401 → 300 (CE 25 eV), *m/z* 417 → 316 (CE 25 eV), *m/z* 385 → 285 (CE 25 eV), and *m/z* 433 → 415 (CE 25 eV), respectively. The lower limits of quantification for gomisin A, gomisin B, gomisin C, gomisin N, deoxyschisandrin, wuweizisu C, and schisandrin were 0.05, 0.005, 0.005, 0.005, 0.005, 0.01, and 0.005 mg/mL, respectively. The inter-assay precision values for all analytes were less than 15.0%.

Results and Discussion

We observed that Schisandra lignan components potently inhibited the CYP3A enzyme, resulting in metabolic drug interactions. Gomisin B and gomisin C, with methylenedioxyphenyl and bulky groups at position 6, most strongly inhibited CYP3A metabolism, with IC_{50} values as low as 0.19-0.62 mM, which was much lower than those of the other P450s.[9,12] In addition, lignans having one methylenedioxyphenyl group, such as gomisin A, B, C, and N, potently inhibit the CYP3A enzyme in a time- and NADPH-dependent manner through the metabolite-intermediate complexes.[8] Wuzhi capsule, a commercially available Chinese medicine composed of Schisandra extracts, increased plasma concentrations of tacrolimus in combination with tacrolimus in patients who underwent liver transplantation.[13]

Over 20 traditional herbal medicines composed of Schisandra extracts have been documented in the Pharmacopoeia of Korea (https://www.law.go.kr/LSW/admRulLsInfoP.do?admRulSeq=2000000021929). With the increasing consumption of dietary supplements, evaluating their potential to cause HMDIs is crucial. However, reports on the potential of dietary Schisandra supplements to inhibit CYP3A activity are limited. Therefore, we evaluated the CYP3A-inhibitory potential of four dietary Schisandra supplements using HLMs. The addition of commercial dietary Schisandra supplements inhibited microsomal CYP3A-mediated midazolam 1'-hydroxylase activity. The inhibitory potential against CYP3A enzyme followed the order: 'Nature's Way > Swanson > Planetary Herbals ≅ Only Natural (Figure 2).' At a concentration of 0.05 mg/mL, extracts of Schisandra supplement from Nature's Way inhibited CYP3A activity by 93.9%.

Four dietary Schisandra supplements exhibited different CYP3A inhibition potentials. Phytochemical investigations

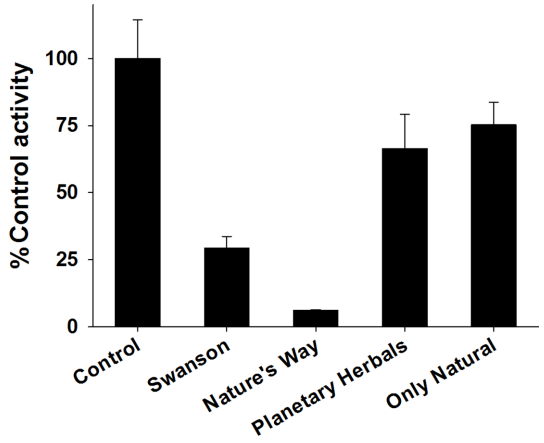

Figure 2. Inhibition of human CYP3A-mediated midazolam 1'-hydroxylation activity by commercial dietary Schisandra supplements. Human liver microsomes (0.25 mg/mL) were incubated with 0.1 mM midazolam and dietary Schisandra supplements (0.05 mg/mL). Data shown are averages of triplicate experiments (n=3).

have showed that dibenzocyclooctadiene lignan contents are quite different according to the variety of Schisandra, cultivation area, and cultivation time. For example, the gomisin C contents in Schisandra chinensis were ca. 20-fold higher than those in Schisandra sphenanthera.[14] In addition, in the same varieties of Schisandra chinensis, the contents of the 10 lignans varied widely among the samples from different districts.[14] Thus, we analyzed the major omija lignan components (gomisin A, gomisin B, gomisin C, gomisin N, deoxyshisandrin, schisandrin, and wuweizisu C) in each product using LC-MS/MS[11] to elucidate the differences in the CYP3A inhibition potential of the four products (Figure 3). The mean correlation coefficient (r^2) of the calibration curves were over 0.984. The limit of quantification for gomisin A, gomisin B, gomisin C, gomisin N, deoxyschisandrin, schisandrin, and wuweizisu C were 50, 5, 5, 5, 5, 5, and 10 ng/mL, respectively. Information on the contents of seven omija lignans in dietary Schisandra supplements is presented in Table 2.

We previously reported that gomisin B and gomisin C strongly inhibit CYP3A-mediated midazolam 1'-hydroxylation activity, with IC_{50} values of 0.42 and 0.30 mM, respectively.[9] The inhibitory effect of gomisin A, gomisin N, deoxyschisandrin, schisandrin, and wuweizisu C on CYP3A activity was 7.4-fold lower than that of gomisin B and gomisin C.[9,15] Nature's Way, which exhibited the strongest inhibitory effect on CYP3A, had the highest contents of gomisin B and gomisin C (Figure 4), which had the strongest inhibitory potential against CYP3A activity. Swanson, which had the second highest gomisin B and gomisin C contents, inhibited the CYP3A activity second. Planetary Herbals and Only Natural, which had a

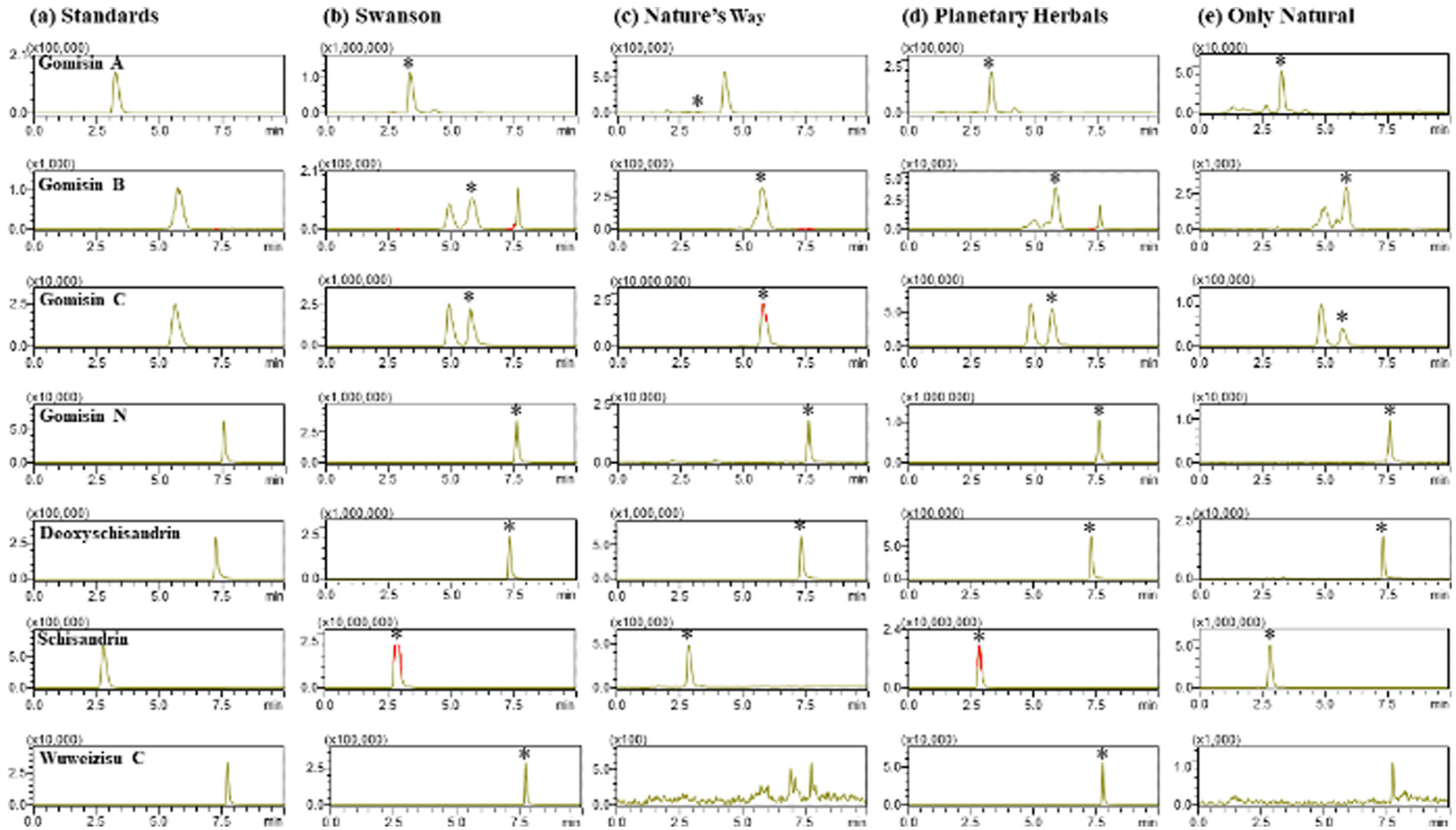

Figure 3. Representative selected reaction monitoring chromatograms of seven dibenzocyclooctadiene lignan standards (a) and dietary Schisandra supplements extracts from Swanson (b), Nature's Way (c), Planetary Herbals (d), and Only Natural (e). Gomisin A (3.25 min), gomisin B (5.79 min), gomisin C (5.64 min), gomisin N (7.58 min), deoxyschisandrin (7.26 min), schisandrin (2.83 min), and wuweizisu C (7.74 min) were monitored in positive electrospray ionization mode.

Table 2. Contents of seven dibenzocyclooctadiene lignans in four dietary Schisandra supplements (n=3).

Lignans Sample	Contents (μg/mg samples)						
	Gomisin A	Gomisin B	Gomisin C	Gomisin N	Deoxyschisandrin	Schisandrin	Wuweizisu C
Swanson	3.167±0.009	0.900±0.002	0.400±0.002	3.300±0.006	0.800±0.001	5.300±0.014	0.233±0.001
Nature's Way	0.050±0.013	3.867±0.002	6.667±0.002	0.020±0.004	4.533±0.001	0.050±0.009	ND*
Planetary Herbals	0.405±0.006	0.129±0.001	0.061±0.001	0.515±0.004	0.132±0.001	0.825±0.003	0.029±0.001
Only Natural	0.120±0.012	0.010±0.002	0.010±0.002	0.010±0.001	0.004±0.001	0.330±0.089	ND*

ND*: not detected

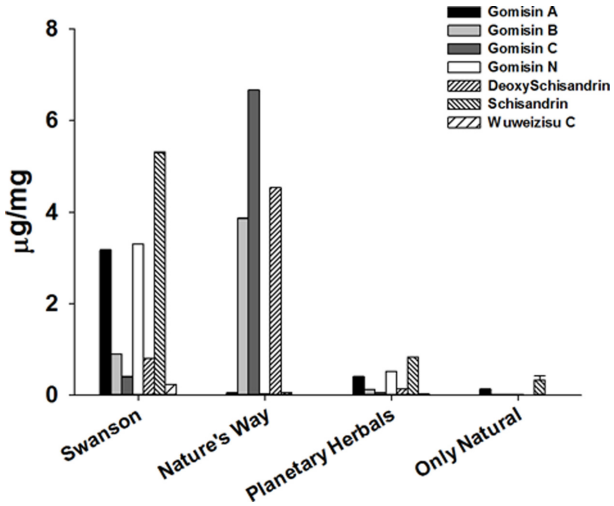

Figure 4. Contents of seven dibenzocyclooctadiene lignans in four dietary Schisandra supplements, measured by liquid chromatography-tandem mass spectrometry. Data shown are averages of triplicate experiments (n=3).

substantially low content of the seven lignans, had negligible inhibitory effects against the CYP3A enzyme.

Schisandra extracts reportedly altered the pharmacokinetics of CYP3A substrate drugs.[8] After oral administration (1.89 mg/kg) of cyclosporine A with co-administration of a Wuzhi tablet (containing 7.5 mg gomisin C/tablet; dose 250 mg/kg), the area under the curve and C_{max} values of cyclosporin A were significantly increased by 293.1% and 84.1%, respectively.[16] Furthermore, oral Wuzhi capsules (containing 11.25 mg deoxyschisandrin/capsule) significantly increased the oral bioavailability of tacrolimus through CYP3A inhibition in healthy individuals.[17] Dietary Schisandra supplements from Nature's Way contained 4.48 mg gomisin B, 7.74 mg gomisin C, and 5.26 mg deoxyschisandrin per servings, which are similar to the contents in Wuzhi tablets (7.5 mg gomisin C/tablet) and capsules (11.25 mg deoxyschisandrin/capsule). Therefore, Nature's Way supplements might potentially cause HMDI with drugs predominantly metabolized by the CYP3A enzyme *in vivo*.

Conclusions

In conclusion, we evaluated the CYP3A inhibitory potential of four dietary Schisandra supplements in human liver microsomes. Dietary supplement from Nature's Way strongly inhibited CYP3A-mediated midazolam 1'-hydroxylase activity. This product contained high levels of gomisin B and gomisin C, which exhibited the strongest inhibitory potential against CYP3A activity. In vivo studies investigating the pharmacokinetic interactions between dietary supplement from Nature's Way and CYP3A substrates are required to determine the clinical relevance of these interactions.

Acknowledgements

This research was supported by Kyungpook National University Research Fund, 2021.

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