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Sulfate Conjugation of Daphnetin by the Human Cytosolic Sulfotransferases

Zhengyang Han^a, Yuecheng Xi^a, Lijun Luo^{a,b}, Chunyang Zhou^{a,b}, Katsuhisa Kurogi^{a,c}, Yoichi Sakakibara^c, Masahito Suiko^c, and Ming-Cheh Liu^{a,*}

^aDepartment of Pharmacology, College of Pharmacy and Pharmaceutical Sciences, University of Toledo Health Science Campus, Toledo, OH 43614 USA

^bSchool of Pharmacy, North Sichuan Medical College, Nanchong, Sichuan, China

^cDepartment of Biochemistry and Applied Biosciences, University of Miyazaki, Miyazaki 889-2192 Japan

Abstract

Ethnopharmacological relevance—In Turkey, daphnetin-containing *Daphne oleoides* is used as a folk medicine for treating rheumatic pain and lumbago. A daphnetin-containing traditional Chinese medicine tablet, named Zushima-Pian, is available in China for treating rheumatoid arthritis. The present study aimed to investigate the metabolism of daphnetin through sulfation in cultured human cells and to identify the human cytosolic sulfotransferase(s) (SULT(s)) that is(are) capable of mediating the sulfation of daphnetin.

Materials and methods—Cultured HepG2 human hepatoma cells and Caco-2 human colon carcinoma cells were labeled with [³⁵S]sulfate in the presence of different concentrations of daphnetin. Thirteen known human SULTs, previously expressed and purified, as well as cytosols of human kidney, liver, lung, and small intestine, were examined for daphnetin-sulfating activity using an established sulfotransferase assay.

Results—[³⁵S]sulfated daphnetin was found to be generated and released by HepG2 cells and Caco-2 cells labeled with [³⁵S]sulfate in the presence of daphnetin. Among the 13 known human SULTs, SULT1A1, SULT1A2, SULT1A3, SULT1B1, and SULT1C4 displayed significant sulfating activity toward daphnetin. Of the four human organ samples later tested, small intestine and liver cytosols displayed considerably higher daphnetin-sulfating activity than those of lung and kidney.

Conclusion—The results derived from the present study showed unequivocally that daphnetin could be sulfated in cultured human cells and by purified human SULT enzymes as well as human

*Corresponding Author: Ming-Cheh Liu, Ph.D., Professor, Department of Pharmacology, College of Pharmacy and Pharmaceutical Sciences, University of Toledo Health Science Campus, 3000 Arlington Avenue, Toledo, OH 43614 USA, Tel: (419) 383-1918, Fax: (419) 383-1909, ming.liu@utoledo.edu.

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organ cytosols. The information obtained provided a basis for further studies on the metabolism of daphnetin through sulfation in vivo.

Graphical Abstract

Keywords

Daphnetin; *Daphne papyracea*; sulfation; cytosolic sulfotransferase; SULT

1. Introduction

Daphnetin, 7,8-Dihydroxycoumarin, is a catecholic plant-derived compound which was first isolated from *Daphne papyracea*, a shrub belonging to the family Thymelaeaceae [Nasipuri and Ramstad, 1973]. As a biologically-active compound, daphnetin has been shown to exhibit multiple pharmacological effects, including analgesic [Jiang et al., 1984], anti-malarial [Yang et al., 1992], anti-inflammatory [Fylaktakidou et al., 2004], anticancer [Finn et al., 2004], anti-arthritic [Gao et al., 2008], and anti-pyretic [Du et al., 2014] activities. In Turkey, daphnetin-containing *Daphne oleoides* is used as a folk medicine for treating rheumatic pain and lumbago [Ye ilada et al., 1995]. A daphnetin-containing traditional Chinese medicine tablet, named Zushima-Pian, is available for treating rheumatoid arthritis [Gao et al., 2008].

Only limited information, however, is currently available with regard to the pharmacokinetics of daphnetin. Analysis of perfusion fluids of duodenum, jejunum and ileum of rats administered with daphnetin revealed the presence of sulfated metabolites, indicating that the cytosolic sulfotransferase (SULT)-mediated sulfation may play a role in the metabolism of daphnetin in vivo [Shan et al., 2011]. Whether daphnetin sulfation may occur in humans, however, remains unknown. In humans, there are thirteen distinct SULTs that have been classified into four major SULT families [Blanchard et al., 2004].

The current study was designed to examine the occurrence of daphnetin sulfation in cultured human cells and to identify the human cytosolic sulfotransferase(s) (SULT(s)) that is(are) responsible for the sulfation of daphnetin. Moreover, cytosols of major human organs were also examined to verify the presence of daphnetin-sulfating activity in vivo.

2. Materials and Methods

2.1 Materials

Daphnetin, adenosine 5'-triphosphate (ATP), 3'-phosphoadenosine-5'-phosphosulfate (PAPS), N-2-hydroxylpiperazine-N-2-ethanesulfonic acid (HEPES), dithiothreitol (DTT), minimum essential medium (MEM), fetal bovine serum(FBS), penicillin G, streptomycin sulfate, and silica gel TLC plates were products of Sigma Chemical Company (St. Louis, MO). PAP[³⁵S] was synthesized from ATP and carrier-free [³⁵S]sulfate using the

bifunctional human PAPS synthetase as described previously [Yanagisawa et al., 1998]. Recombinant human SULTs were prepared as previously described [Sakakibara et al., 1998a; Sakakibara et al., 1998b; Sakakibara et al., 2002; Pai et al., 2002; Suiko et al., 2002]. HepG2 human hepatoma cells (ATCC HB-8065) and Caco-2 human colon adenocarcinoma cells (ATCC HTB-37) were from American Type Culture Collection (Manassas, VA). Pooled human lung, liver, small intestine, and kidney cytosols were purchased from XenoTech, LLC. All other chemicals were of the highest grade commercially available.

2.2 Metabolic labeling of HepG2 cells and Caco-2 cells

HepG2 cells and Caco-2 cells were routinely maintained, under a 5% CO₂ atmosphere at 37°C, in MEM supplemented with 10% FBS, penicillin G (30 µg/mL) and streptomycin sulfate (50 µg/mL). Confluent cells grown in individual wells of a 24-well culture plate, pre-incubated in sulfate-free (prepared by omitting streptomycin sulfate and replacing magnesium sulfate with magnesium chloride) MEM without FBS for 4 hours, were labeled with 0.25 mL aliquots of the same medium containing [³⁵S]sulfate (0.3 mCi/mL) plus different concentrations (0, 1, 5, 10, 25, and 50 µM) of daphnetin. At the end of an 18-hour labeling period, the labeling media were collected, spin filtered to remove high-molecular weight [³⁵S]sulfated macromolecules, and subjected to silica gel TLC analysis for [³⁵S]sulfated daphnetin using acetic acid/n-butanol (2:1 by volume) as the solvent system.

2.3 Sulfotransferase assay

The sulfating activity of the recombinant human SULTs was assayed using PAP[³⁵S] as the sulfate group donor. The standard assay mixture, in a final volume of 20 µL, contained 50 mM of HEPES buffer at pH 7.0, 1 mM DTT, 14 mM PAP[³⁵S], and 10 µM daphnetin. The reaction was started by the addition of the SULT enzyme, allowed to proceed for 10 min at 37°C, and terminated by placing the thin-walled tube containing the assay mixture on a heating block, pre-heated to 100°C, for 3 min. The precipitates were cleared by centrifugation at 13,000 rpm for 3 min, and the supernatant was subjected to the analysis of [³⁵S]sulfated daphnetin using the TLC procedure as described above. Upon completion of TLC, the TLC plate was air dried and autoradiographed using an X-ray film. The radioactive spot corresponding to the sulfated daphnetin was located, cut out, and eluted in 0.5 mL water in a glass vial. Afterwards, 2 mL of the Ecolume scintillation liquid was added to each vial, mixed thoroughly and the radioactivity therein was counted by using a liquid scintillation counter.

3. Results and Discussion

As a first step toward clarifying the possible metabolism of daphnetin through sulfation in humans, a metabolic labeling experiment was performed using HepG2 human hepatoma cells and Caco-2 human colon adenocarcinoma cells. These two cell lines were chosen because they have previously been reported to express a number of SULT enzymes (SULT1A1, SULT1A2, SULT1A3, SULT1E1, and SULT2A1 for HepG2 cells [Westerink and Schoonen, 2007] and SULT1A1, SULT1A2, SULT1A3, SULT1B1, SULT1C2, SULT1C4, and SULT2A1 for Caco-2 cells [Teubner et. al., 2007]). Results shown in Figure

1 revealed clearly the generation and release of [^{35}S]sulfated daphnetin in a concentration-dependent manner by both HepG2 and Caco-2 cells.

An ensuing question is which of the thirteen known human SULTs is(are) responsible for the sulfation of daphnetin. As shown in Table 1, a systematic analysis revealed that five (SULT1A1, SULT1A2, SULT1A3, SULT1B1, and SULT1C4) of the thirteen human SULTs displayed sulfating activity towards daphnetin. Among these five enzymes, SULT1A1 showed the strongest daphnetin-sulfating activity, followed by SULT1A3, SULT1C4, SULT1A2, and SULT1B1. It is noted that previous studies have demonstrated abundant expression of SULT1A1 in the liver and small intestine [Riches et al., 2009]; whereas SULT1A3 has been shown to be heavily expressed in the small intestine [Riches et al., 2009]. SULT1C4, on the other hand, has been shown to be expressed at much higher levels in fetal and possibly neonatal tissues, as compared with adult tissues [Sakakibara et al., 1998b]. Of the two remaining (minor) daphnetin-sulfating SULTs, SULT1B1 has been shown to be expressed in human liver [Riches et al., 2009]; while the tissue expression of SULT1A2 remains obscure [Nowell et al., 2005].

To verify the presence of daphnetin-sulfating activity in human organs, enzymatic assays were performed using cytosols prepared from human liver, lung, kidney, and small intestine. Activity data obtained are compiled in Table 2. Of the four human organ samples tested, small intestine and liver cytosols exhibited much stronger sulfating activities than did cytosols prepared from lung and kidney. These results indicated that liver and intestine are major organs involved in the sulfation of daphnetin in human body. It is possible that upon oral absorption, daphnetin may be subjected to first-pass metabolism by sulfation in the liver and small intestine. Whether sulfated daphnetin, compared with its unsulfated parent compound, may (still) manifest biological activities remains an open question. This latter question warrants additional investigation since as described above, daphnetin has been associated with a number of pharmacological effects, particularly the anti-inflammatory and anticancer activities [Fylaktakidou et al., 2004; Finn et al., 2004]. It is worthwhile mentioning that a number of plant-derived products, including catechin, epicatechin, eriodictyol, gingerol, hesperetin, kaempferol, myricetin, quercetin, etc., have also been shown to be subjected to sulfation by the SULT enzymes [Pai et al., 2001; Huang et al., 2009; Lu et al., 2016]. Sulfation as mediated by the SULTs, therefore, may represent a major pathway for the biotransformation of many other plant-derived compounds.

4. Conclusions

Collectively, the results from the current study provided for the first time evidence supporting the possible metabolism of daphnetin through sulfation in humans. Further studies are warranted in order to fully elucidate the role of SULT-mediated sulfation in the pharmacokinetics and pharmacodynamics of daphnetin in humans.

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Abbreviations

SULT	cytosolic sulfotransferase
TLC	thin-layer chromatography
PAPS	3'-phosphoadenosine-5'-phosphosulfate.

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Figure 1.

Analysis of [^{35}S]sulfated products generated and released by HepG2 cells and Caco-2 cells labeled with [^{35}S]sulfate in the presence of daphnetin. The figure shows the autoradiograph taken from the TLC plate at the end of the TLC analysis. Confluent cells were incubated in labeling media containing, respectively, 1, 5, 10, 25, and 50 μM (corresponding to lanes 1–5) of daphnetin for 18 hours. C refers to the control labeling medium without daphnetin. E refers to [^{35}S]sulfated daphnetin generated enzymatically using human SULT1A1. The arrows indicate radioactive spots corresponding to [^{35}S]sulfated daphnetin.

Table 1

A) Specific activities of purified human SULT1A1, SULT1A2, SULT1A3, and SULT1C4 with daphnetin as a substrate^a

Substrate	Specific Activity (nmol/min/mg)			
	SULT1A1	SULT1A2	SULT1A3	SULT1C4
Daphnetin	30.37 ± 0.36	1.89 ± 0.04	14.15 ± 0.09	0.93 ± 0.05
				13.09 ± 0.19

B) Specific activities of human lung, liver, kidney, and small intestine cytosols with daphnetin as a substrate^a

Substrate	Specific Activity (pmol/min/mg)			
	Lung	Liver	Kidney	Small Intestine
Daphnetin	29.67 ± 1.53	113.33 ± 5.86	8.00 ± 0.01	297.33 ± 15.57

^a Specific activity refers to pmol substrate sulfated/min/mg protein. Data represent mean ± S.D. derived from three determinations.