Pergamon

Biochemical Pharmacology, Vol. 50, No. 5, pp. 731–734, 1995.

0006-2952/95 $9.50 + 0.00

0006-2952(95)00190-5

Short Communication

QUERCETIN, A POTENT AND SPECIFIC INHIBITOR OF THE HUMAN P-FORM PHENOLSULFOTRANSFERASE

THOMAS WALLE,* E. ALISON EATON and U. KRISTINA WALLE

Department of Cell and Molecular Pharmacology and Experimental Therapeutics, Medical University of South Carolina, Charleston, SC 29425, U.S.A.

(*Received* 7 *December* 1994; *accepted* 14 *March* 1995)

Abstract—The natural product quercetin was a potent inhibitor of the human P-form phenolsulfotransferase with an IC_{50} value of 0.10 ± 0.03 μM (mean ± SEM; N = 5), which was three to four orders of magnitude more potent than its inhibition of other human sulfotransferases. The inhibition was noncompetitive with a K_i value of 0.10 μM. The potency and mechanism of this inhibition appear similar to those of the current standard P-form inhibitor, 2,6-dichloro-4-nitrophenol. Among other flavonoids examined, kaempferol was found to have an IC_{50} value of 0.39 ± 0.07 μM, naringenin 10.6 ± 1.6 μM and naringin 265 ± 90 μM (N = 3). These observations suggest the potential for clinically important pharmacologic and toxicologic interactions by flavonoid-containing foods and beverages.

Key words: quercetin; sulfotransferase; enzyme inhibition; flavonoids; kaempferol; naringenin

Sulfation (sulfonation) is one of the major phase II conjugation reactions for drugs and environmental chemicals as well as for endogenous compounds such as steroids and monoamine neurotransmitters [1–3]. This biotransformation reaction is usually a detoxification process, leading to greatly enhanced renal excretion of the highly charged sulfuric acid ester conjugates formed. However, sometimes labile, chemically reactive intermediates are formed, which can undergo DNA binding, leading to mutagenicity and carcinogenicity [4–7]. Thus, altered activity of the enzymes responsible for these reactions, i.e. the STs† [1–3], could have major pharmacologic as well as toxicologic implications.

Numerous reports have shown inhibition of the human STs by a variety of drugs and food and beverage constituents [8–11]. Such inhibition may affect all of the known STs [1–3], i.e. the P and M forms of PST, DHEA-ST, and estrogen ST, although isoform-selectivity has been observed with some inhibitors. Particularly potent inhibitors appear to be present in natural products such as red wine, although their chemical structures remain unknown [8].

In the present study, we report for the first time on the inhibitory effect of quercetin and other flavonoids (Fig. 1) on the human STs. The potency of quercetin was high, and the effect was specific for the P-form PST.

Materials and Methods

Materials. Quercetin, kaempferol, naringin, naringenin, *p*-nitrophenol, *p*-nitrophenyl sulfate, DHEA, dopamine, estrone and ethinyl estradiol were purchased from Sigma (St. Louis, MO); DCNP was obtained from the Aldrich Chemical Co. (Milwaukee, WI) and [^{35}S]PAPS (sp. act. 1.0 to 1.5 Ci/mmol) from Du Pont New England Nuclear (Wilmington, DE). Cytosol from human liver (Liver Tissue Procurement and Distribution System, University of Minnesota, Minneapolis, MN) was prepared as previously described [12–14]. Partially purified P-form PST was prepared from one of the human liver cytosols by DEAE-Sepharose column chromatography [12–14]. Dithiothreitol and BSA were added as preservatives, and the enzyme was stored at −80°.

Incubations. Cytosol or partially purified P-form PST with *p*-nitrophenol (4 μM), dopamine (40 μM), DHEA (5 μM) and estrone (5 μM) was incubated at 37° for 30 min in 33 mM Tris buffer, pH 7.4, with BSA (0.0625%) and dithiothreitol (8 mM) and [^{35}S]PAPS (0.4 μM) as the cosubstrate [12–14]. As increasing the PAPS concentration

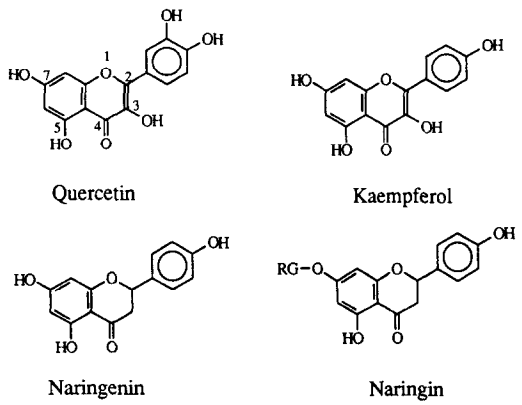

Fig. 1. Chemical structures of the flavonoids used in the study. RG in naringin indicates a rhamnoglucoside.

* Corresponding author: Thomas Walle, Ph.D., Department of Cell and Molecular Pharmacology and Experimental Therapeutics, Medical University of South Carolina, 171 Ashley Ave., Charleston, SC 29425. Tel. (803) 792-2471; FAX (803) 792-2475.

† Abbreviations: ST, sulfotransferase; P form, phenol form; M form, monoamine form; PST, phenolsulfotransferase; DHEA, dehydroepiandrosterone; DCNP, 2,6-dichloro-4-nitrophenol; and PAPS, 3′-phosphoadenosine-5′-phosphosulfate.

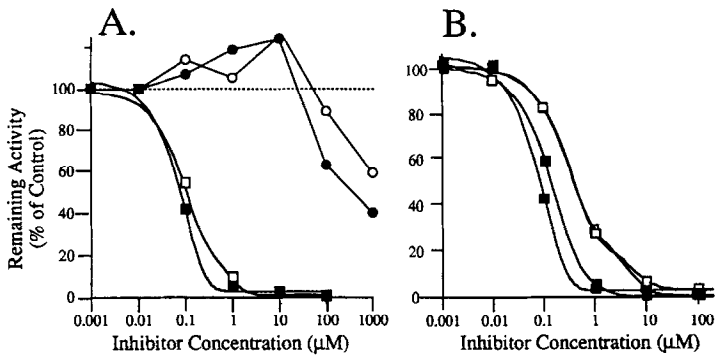

Fig. 2. (A) Effect of quercetin on the sulfation of *p*-nitrophenol (■), dopamine (●) and DHEA (○) by the human liver cytosol. Shown for comparison is the effect of DCNP on the sulfation of *p*-nitrophenol (□). Each concentration–effect curve is the mean of 3–5 determinations, using three different livers. Control activity was 308 ± 109 pmol·min^{-1}·(mg protein)$^{-1}$ (mean ± SEM; N = 3). (B) Effects of quercetin (■) and DCNP (□) on the sulfation of *p*-nitrophenol by the partially purified P-form PST. Results are shown for two experiments with each inhibitor. The corresponding IC_{50} values are shown in Table 1.

from 0.4 to 10 μM did not influence our results, we chose to use the lower concentration commonly employed in many studies. With *p*-nitrophenol as substrate, the amount of cytosol (about 10 μg protein) and partially purified P-form PST used produced about 2.5 pmol *p*-nitrophenyl sulfate per minute and incubate. Preincubations with inhibitors (quercetin, kaempferol, naringin, naringenin and DCNP) were for 5 min at 37°. [^{35}S]PAPS and protein were precipitated with barium hydroxide and zinc sulfate [15], and an aliquot of the supernatant was counted by liquid scintillation spectrometry. Incubates were done in duplicate. Blank incubates, i.e. without substrate, were run for each inhibitor concentration (0 and 0.01 to 1000 μM), and these counts were subtracted from the corresponding substrate-containing incubates.

The validity of the precipitation assay described above was confirmed by HPLC analysis. Incubates with *p*-nitrophenol (4 μM) as the substrate and quercetin as inhibitor were precipitated as above; part of the supernatant was counted directly and part was analyzed for ^{35}S-labeled *p*-nitrophenyl sulfate by HPLC after the addition of 5 μg of unlabeled *p*-nitrophenyl sulfate [16]. The samples were run on an ODS-1 150 × 4.6 mm column (Phenomenex, Torrance, CA) with a mobile phase of 15% acetonitrile in 0.05 M ammonium acetate buffer (pH 4) at 0.8 mL/min. The *p*-nitrophenyl sulfate peak at a retention time of 7 min was visualized at 280 nm and quantified with a Flo-One radiometric detector (Packard Instruments, Meriden, CT).

Data analysis. The IC_{50} values for the concentration–activity curves from individual experiments were derived with UltraFit (Biosoft, Cambridge, U.K.), using an equation for double exponential decay with offset. The mechanism of inhibition of P-form PST by quercetin and its K_i value were derived graphically from the Lineweaver–Burk plot (1/V versus 1/S) and the replot of the 1/V axis intercept versus inhibitor concentration, respectively [17].

Results

The effects of quercetin and DCNP on the sulfation of *p*-nitrophenol, the prototype substrate for the human P-form PST, by the cytosol of human liver are shown in Fig. 2A. Each concentration–effect curve represents the mean values from three human livers. Quercetin and DCNP showed virtually identical inhibition curves with IC_{50} values of 0.10 ± 0.03 μM (mean ± SEM) for quercetin and 0.11 ± 0.01 μM for DCNP (Table 1). Figure 2B shows two

Table 1. IC_{50} Values for inhibition of *p*-nitrophenol sulfoconjugation by flavonoids and DCNP

	IC_{50} (μM)	N
Liver cytosol		
Quercetin	0.10 ± 0.03*	5
DCNP	0.11 ± 0.01	3
Kaempferol	0.39 ± 0.07	3
Naringenin	10.6 ± 1.6	3
Naringin	265 ± 90	3
Partially purified P-form PST		
Quercetin	0.10	2
DCNP	0.23	2

In each assay, the incubation was done for 30 min at 37° in the presence of 4 μM *p*-nitrophenol and various concentrations of inhibitor. Control activity was 308 ± 109 pmol·min^{-1}·(mg protein)$^{-1}$ (mean ± SEM; N = 3).
* Mean ± SEM.

experiments each with quercetin and DCNP, using the partially purified P-form PST to catalyze *p*-nitrophenol sulfation. Whereas the inhibition by quercetin was identical to that in the cytosol (Fig. 2A), DCNP appeared to be slightly less potent (Table 1).

To test the isoform-specificity of the inhibition, we also examined the influence of quercetin on the sulfate conjugation by the other known human ST isoforms present in the human liver. Using dopamine as a probe for the M-form PST and DHEA for DHEA-ST, we observed IC_{50} values of about 500–1000 μM (Fig. 2A). The inhibition of the sulfation of estrone and ethinyl estradiol as a measure of estrogen ST required quercetin concentrations in excess of 1000 μM.

To explore the mechanism by which quercetin inhibits the sulfation catalyzed by the P-form PST, we used the partially purified enzyme. Preliminary experiments demonstrated that DCNP was an inhibitor mostly resembling the noncompetitive type. A Lineweaver–Burk plot of the quercetin data is shown in Fig. 3A, where the *x*-axis intercept gives the K_m value for *p*-nitrophenol (about

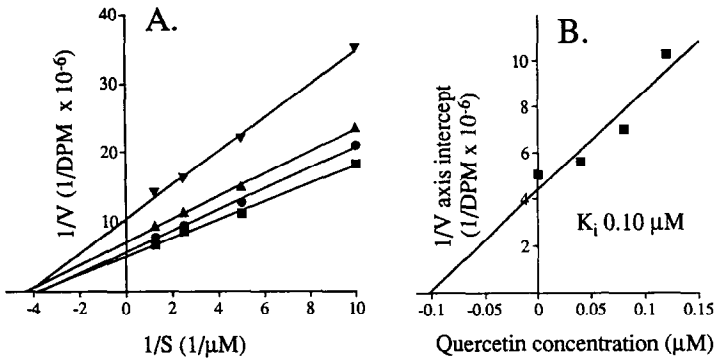

Fig. 3. Quercetin inhibition of *p*-nitrophenol sulfation catalyzed by partially purified P-form PST. (A) Lineweaver–Burk plot. The quercetin concentrations were 0 μM (■), 0.04 μM (●), 0.08 μM (▲) and 0.12 μM (▼). (B) Replot from panel A.

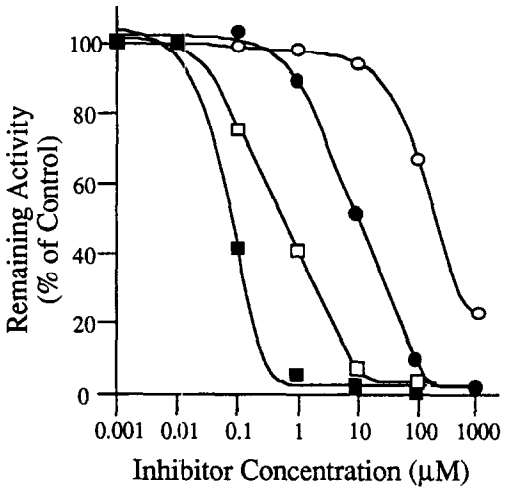

Fig. 4. Effects of quercetin (■), kaempferol (□), naringenin (●) and naringin (○) on *p*-nitrophenol sulfation by the human liver cytosol. Each concentration–effect curve is the mean of 3–5 determinations. The corresponding IC_{50} values are shown in Table 1. Control activity was 308 ± 109 pmol · min^{-1} · (mg protein)$^{-1}$ (mean ± SEM; N = 3).

0.25 μM). This plot demonstrates that quercetin is a noncompetitive inhibitor. The replot of the 1/V axis intercept versus quercetin concentration (Fig. 3B) [17] gave a K_i value of 0.10 μM, i.e. identical to the IC_{50} value. Parallel lines in an Eadie–Scatchard plot (V/S vs V) confirmed that the inhibition was noncompetitive (data not shown).

To determine if the inhibition of the P-form PST by quercetin was common to all flavonoids, we also examined the effects of kaempferol, naringenin and naringin on *p*-nitrophenol sulfation by the human liver cytosol, as shown in Fig. 4. All compounds were inhibitory with IC_{50} values of 0.39 ± 0.07 μM for kaempferol, 10.6 ± 1.6 μM for naringenin, and 265 ± 90 μM for naringin (Table 1).

In the initial experiments using the precipitation assay by Foldes and Meek [15] to determine *p*-nitrophenyl sulfate formation, both quercetin and DCNP inhibited the background by as much as 80%. When this was taken into account, the precipitation assay gave results identical to those obtained by the more specific HPLC assay for the *p*-nitrophenyl sulfate formed [16]. Experiments with cytosol, [^{35}S]PAPS and quercetin (0.01 to 1000 μM) only demonstrated that quercetin was not a substrate for sulfoconjugation under the conditions used.

Discussion

This study has demonstrated potent inhibition of the human P-form PST by the flavonoid type of natural products, in particular quercetin, producing IC_{50} or K_i values as low as 0.10 μM. This inhibition was highly isoform-selective, as quercetin was three to four orders of magnitude less potent in inhibiting the DHEA-ST, the M-form PST, and the estrogen ST.

Previous studies have suggested that other natural product inhibitors of the sulfotransferases may also be selective for the P-form PST [8, 9]. In contrast, food additives show very little selectivity for the various ST isoforms [11]. The very high potency and selectivity of quercetin were similar to the same properties of the industrial chemical DCNP, which together with pentachlorophenol was discovered by Mulder and coworkers to be potent inhibitors [18, 19] and were used subsequently as powerful tools to help distinguish between the P-form PST and other STs [1–3, 20, 21]. The mechanism of inhibition of the P-form PST by quercetin was noncompetitive in nature. This also appeared to be the case with DCNP, similar to a previous study with the human brain enzyme [22]. Quercetin was not a substrate for sulfation under the conditions used and, therefore, may be characterized as a dead-end inhibitor similar to DCNP and pentachlorophenol [22–25]. The lack of sulfation of quercetin and DCNP by the human enzyme appears to be different from the situation in the rat, in which sulfation of both quercetin and DCNP takes place [26, 27].

When extending the studies to several additional flavonoids, it was found that kaempferol, which lacks one of the hydroxyl groups on the 2-phenyl substituent of quercetin (Fig. 1), was 4 times less potent than quercetin. Naringenin, which lacks the 2,3 double bond and the hydroxyl group in the 3-position as compared with kaempferol, was 106 times less potent than quercetin. Naringin, the 7-rhamnoglucoside conjugate of naringenin, showed a further 25-fold decrease in potency. These observations indicate interesting structure–activity relationships for the inhibitory action of flavonoids on the P-form PST. More extensive structural studies may reveal even more potent inhibitors than quercetin, as well as provide

a more detailed picture of the structural requirement for inhibition.

The potential clinical significance of these findings could involve flavonoid-induced inhibition of metabolism of drugs such as acetaminophen and minoxidil, for which sulfation by the P-form PST is essential [28, 29]. This could lead to either an increase, as for acetaminophen, or a decrease, as for minoxidil, in the intensity and duration of the pharmacologic action. The flavonoids are also potentially important chemopreventive agents through inhibition of the activating sulfation step for a number of mutagens and carcinogens [4–7, 30]. For this to occur, the flavonoids, present in a variety of foods and natural product extracts [31, 32] including red wine [8], must be able to produce these effects *in vivo*, analogously to grapefruit juice inhibition of cytochrome P450-catalyzed drug oxidations, an effect proposed to be mediated also by flavonoids [33].

Acknowledgement—This work was supported, in part, by a grant from the National Institutes of Health (GM46000).

REFERENCES

1. Mulder GJ and Jakoby WB, Sulfation. In: *Conjugation Reactions in Drug Metabolism: An Integrated Approach* (Ed. Mulder GJ), pp. 107–161. Taylor & Francis, London, 1990.
2. Weinshilboum RM, Phenol sulfotransferase in humans: Properties, regulation, and function. *Fed Proc* **45**: 2223–2228, 1986.
3. Falany CN, Molecular enzymology of human liver cytosolic sulfotransferases. *Trends Pharmacol Sci* **12**: 255–259, 1991.
4. Surh Y-J and Tannenbaum SR, Activation of the Maillard reaction product 5-(hydroxymethyl)furfural to strong mutagens via allylic sulfonation and chlorination. *Chem Res Toxicol* **7**: 313–318, 1994.
5. Gilissen RAHJ, Hume R, Meerman JHN and Coughtrie MWH, Sulphation of *N*-hydroxy-4-aminobiphenyl and *N*-hydroxy-4-acetylaminobiphenyl by human foetal and neonatal sulphotransferase. *Biochem Pharmacol* **48**: 837–840, 1994.
6. Miller JA, Sulfonation in chemical carcinogenesis. History and present status. *Chem Biol Interact* **92**: 329–341, 1994.
7. Kato R and Yamazoe Y, Metabolic activation of *N*-hydroxylated metabolites of carcinogenic and mutagenic arylamines and arylamides by esterification. *Drug Metab Rev* **26**: 413–430, 1994.
8. Littlewood JT, Glover V and Sandler M, Red wine contains a potent inhibitor of phenolsulphotransferase. *Br J Clin Pharmacol* **19**: 275–278, 1985.
9. Gibb C, Glover V and Sandler M, *In vitro* inhibition of phenolsulphotransferase by food and drink constituents. *Biochem Pharmacol* **36**: 2325–2330, 1987.
10. Bamforth KJ, Dalgliesh K and Coughtrie MWH, Inhibition of human liver steroid sulfotransferase activities by drugs: A novel mechanism of drug toxicity? *Eur J Pharmacol* **228**: 15–21, 1992.
11. Bamforth KJ, Jones AL, Roberts RC and Coughtrie MWH, Common food additives are potent inhibitors of human liver 17α-ethinyloestradiol and dopamine sulphotransferases. *Biochem Pharmacol* **46**: 1713–1720, 1993.
12. Walle T and Walle UK, Stereoselective sulfate conjugation of 4-hydroxypropranolol and terbutaline by the human liver phenolsulfotransferases. *Drug Metab Dispos* **20**: 333–336, 1992.
13. Pesola GR and Walle T, Stereoselective sulfate conjugation of isoproterenol in humans: Comparison of hepatic, intestinal and platelet activity. *Chirality* **5**: 602–609, 1993.
14. Campbell NRC, Van Loon JA and Weinshilboum RM, Human liver phenol sulfotransferase: Assay conditions, biochemical properties and partial purification of isozymes of the thermostable form. *Biochem Pharmacol* **36**: 1435–1446, 1987.
15. Foldes A and Meek JL, Rat brain phenol-sulfotransferase—Partial purification and some properties. *Biochim Biophys Acta* **327**: 365–374, 1973.
16. Shwed JA, Walle UK and Walle T, Hep G2 cell line as a human model for sulfate conjugation of drugs. *Xenobiotica* **22**: 973–982, 1992.
17. Segel IH, *Enzyme Kinetics*. John Wiley, New York, 1975.
18. Mulder GJ and Scholtens E, Phenol sulphotransferase and uridine diphosphate glucuronyltransferase from rat liver *in vivo* and *in vitro*. 2,6-Dichloro-4-nitrophenol as selective inhibitor of sulphation. *Biochem J* **165**: 553–559, 1977.
19. Koster H, Halsema I, Scholtens E, Meerman JHN, Pang KS and Mulder GJ, Selective inhibition of sulfate conjugation in the rat. Pharmacokinetics and characterization of the inhibitory effect of 2,6-dichloro-4-nitrophenol. *Biochem Pharmacol* **31**: 1919–1924, 1982.
20. Rein G, Glover V and Sandler M, Multiple forms of phenolsulfotransferase in human tissues: Selective inhibition by dichloronitrophenol. *Biochem Pharmacol* **31**: 1893–1897, 1982.
21. Walle T and Walle UK, Stereoselective sulfate conjugation of racemic 4-hydroxypropranolol by human and rat liver cytosol. *Drug Metab Dispos* **19**: 448–453, 1991.
22. Whittemore RM, Pearce LB and Roth JA, Purification and kinetic characterization of a phenol-sulfating form of phenol sulfotransferase from human brain. *Arch Biochem Biophys* **249**: 464–471, 1986.
23. Sekura RD, Duffel MW and Jakoby WB, Aryl sulfotransferases. *Methods Enzymol* **77**: 197–206, 1981.
24. Duffel MW and Jakoby WB, On the mechanism of aryl sulfotransferase. *J Biol Chem* **256**: 11123–11127, 1981.
25. Giorgi O and Meek JL, Sulfation of peptides and simple phenols by rat brain phenolsulfotransferase. Inhibition by dichloronitrophenol. *Biochem Pharmacol* **34**: 45–49, 1985.
26. Shali NA, Curtis CG, Powell GM and Roy AB, Sulphation of the flavonoids quercetin and catechin by rat liver. *Xenobiotica* **21**: 881–893, 1991.
27. Seah VMY and Wong KP, 2,6-Dichloro-4-nitrophenol (DCNP), an alternate-substrate inhibitor of phenol-sulfotransferase. *Biochem Pharmacol* **47**: 1743–1749, 1994.
28. Reiter C and Weinshilboum RM, Acetaminophen and phenol: Substrates for both a thermostable and a thermolabile form of human platelet phenol sulfotransferase. *J Pharmacol Exp Ther* **221**: 43–51, 1982.
29. Falany CN and Kerl EA, Sulfation of minoxidil by human liver phenol sulfotransferase. *Biochem Pharmacol* **40**: 1027–1032, 1990.
30. Chou H-C, Lang NP and Kadlubar FF, Metabolic activation of *N*-hydroxy arylamines and *N*-hydroxy heterocyclic amines by human sulfotransferase(s). *Cancer Res* **55**: 525–529, 1995.
31. Havsteen B, Flavonoids, a class of natural products of high pharmacological potency. *Biochem Pharmacol* **32**: 1141–1148, 1983.
32. Hertog MGL, Hollman PCH, Katan MB and Kromhout D, Intake of potentially anticarcinogenic flavonoids and their determinants in adults in The Netherlands. *Nutr Cancer* **20**: 21–29, 1993.
33. Bailey DG, Arnold JMO and Spence JD, Grapefruit juice and drugs: How significant is the interaction? *Clin Pharmacokinet* **26**: 91–98, 1994.